2015

OUTCOMES ASSOCIATED WITH BLOOD COMPONENT TRANSFUSION IN ADULT TRAUMA PATIENTS

Allison R. Jones
University of Kentucky, allisonjones3684@gmail.com

STUDENT AGREEMENT:

I represent that my thesis or dissertation and abstract are my original work. Proper attribution has been given to all outside sources. I understand that I am solely responsible for obtaining any needed copyright permissions. I have obtained needed written permission statement(s) from the owner(s) of each third-party copyrighted matter to be included in my work, allowing electronic distribution (if such use is not permitted by the fair use doctrine) which will be submitted to UKnowledge as Additional File.

I hereby grant to The University of Kentucky and its agents the irrevocable, non-exclusive, and royalty-free license to archive and make accessible my work in whole or in part in all forms of media, now or hereafter known. I agree that the document mentioned above may be made available immediately for worldwide access unless an embargo applies.

I retain all other ownership rights to the copyright of my work. I also retain the right to use in future works (such as articles or books) all or part of my work. I understand that I am free to register the copyright to my work.

REVIEW, APPROVAL AND ACCEPTANCE

The document mentioned above has been reviewed and accepted by the student's advisor, on behalf of the advisory committee, and by the Director of Graduate Studies (DGS), on behalf of the program; we verify that this is the final, approved version of the student's thesis including all changes required by the advisory committee. The undersigned agree to abide by the statements above.

Allison R. Jones, Student

Dr. Susan K. Frazier, Major Professor

Dr. Terry A. Lennie, Director of Graduate Studies

OUTCOMES ASSOCIATED WITH BLOOD COMPONENT TRANSFUSION IN ADULT TRAUMA PATIENTS

DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Nursing
at the University of Kentucky

By
Allison Roenker Jones

Lexington, Kentucky

Director: Dr. Susan K. Frazier, Associate Professor of Nursing

Lexington, Kentucky

2015

ABSTRACT OF DISSERTATION

OUTCOMES ASSOCIATED WITH BLOOD COMPONENT TRANSFUSION IN ADULT TRAUMA PATIENTS

The purpose of this dissertation was to evaluate outcomes associated with blood component (BC) transfusion in adult trauma patients. Specific aims were to: 1) explore the relationship between traumatic injury, hemorrhage, and BC transfusion, focusing on consequences of the component storage lesion through presentation of a conceptual model; 2) systematically review research literature comparing outcomes of massively transfused major trauma patients based on ratios of BCs received; 3) evaluating the relationship between type of blood transfusion trauma patients received (whole blood versus BCs) and mortality likelihood after controlling for demographic and clinical variables; 4) evaluating the relationship between volume and ratio of BCs transfused to trauma patients and development of inflammatory complications (ICs) after controlling for demographic and clinical variables.

Specific aim one was addressed through the development of a conceptual model, depicting the current state of knowledge regarding the storage lesion, and short-/long-term outcomes of traumatic injury, hemorrhage, and blood transfusion. The second specific aim was addressed through a systematic review of studies that grouped critically injured, massively transfused patients based on ratios of BCs they received, and compared clinical outcomes among groups. Findings from this analysis revealed increased survival likelihood with massive transfusion of BCs in a 1:1:1 (packed red blood cells [PRBCs], fresh frozen plasma [FFP], platelets [PLTs]) fashion. The third specific aim involved a secondary analysis of the National Trauma Data Bank to evaluate the relationship between type of transfusion trauma patients received (whole blood versus BCs) and mortality. Patients who received BCs experienced a higher mortality likelihood compared with those who received whole blood. The fourth specific aim was addressed through a secondary analysis of the Inflammation and Host Response to Injury Trauma-Related Data Base, to evaluate the relationship between volume and ratio of BCs transfused and development of ICs in patients with major trauma. Findings revealed that total transfused volume of PRBCs, injury severity, and comorbidities were associated with development of ICs. There were no differences in time to complication between PRBCs:FFP or PRBCs:PLTs ratio groups.

KEYWORDS: Blood Transfusion, Blood Component, Trauma, Ratio, Storage Lesion

Allison R. Jones
Student's Signature

March 23, 2015
Date

OUTCOMES ASSOCIATED WITH BLOOD COMPONENT TRANSFUSION IN ADULT TRAUMA PATIENTS

By

Allison Roenker Jones

Dr. Susan K. Frazier
Director of Dissertation

Dr. Terry A. Lennie
Director of Graduate Studies

March 23, 2015

This dissertation is dedicated to my parents,
Kenneth and Janice Roenker.

You have lived lives dedicated to education and service to others.
Thank you for not only teaching me the value of education and
the importance of giving of yourself, but for inspiring me to
combine them into a passion and profession.

I could never properly express my gratitude for all you have done
and all of your support, but I sincerely hope that this dissertation
serves as a token of my appreciation and makes you proud.

All my love, Al.

ACKNOWLEDGMENTS

This work would not have been possible without the guidance and support of my committee. Dr. Susan Frazier, your constant encouragement, enthusiasm, and excitement for this dissertation have helped me realize my greatest potential and envision the endless possibilities this research provides for future endeavors. You have allowed me to see research as something impactful and meaningful. Thank you. Dr. Terry Lennie, I want to thank you for continuously challenging me throughout this process, and for being determined to make this work the best it could be. Dr. Debra Moser, I thank you for your honesty and your drive to help me succeed. Dr. Patricia "Mama" Howard, thank you for providing constant support in both my bedside and academic nursing careers; your endless encouragement and aid in all areas has been invaluable. Dr. Heather Bush, you are my statistics hero! You made a topic I found extremely difficult completely understandable – you have a gift, and I thank you for sharing it. You have all allowed me to continue to push myself in new directions, and I look forward to the next steps.

To my husband, Pete, I don't know that I could ever truly express my gratitude for every kind of support you have offered over the past five years. You have loved me unconditionally and tolerated me when I least deserved it. We have been blessed with an amazing opportunity to grow personally and professionally together through our graduate programs, and I wouldn't trade these years for anything. I love you, and I am so excited to see what is to come.

To my parents, I would not have been able to complete this degree or survive the past five years without your constant encouragement and support. I cannot express to you what this has meant to me. To Anne, thank you for allowing me to continue to be your baby sister. You keep me in touch with my lighter side and remind me that my crazy is a good kind of crazy. I love you for that. To Christy, I love so much being able to share the highs and lows that is a love of medicine and research with you. It is something I never anticipated, but have so much enjoyed developing this relationship with you. To my Brady man, my Reese cup, my Peach, and my Bubs – the greatest blessing of this journey has been the time it has allowed me to spend with you during your first few years in this world. You've helped me to play and laugh and be a child again when I most needed it. I could never thank you enough. Finally, to my amazing in-laws, Matt, Kevin, the Hicks, Jones, and Kamuf families, you have loved and supported me more than I could have asked for throughout this process. I am eternally grateful for your presence in my life.

To all of my friends and extended family – I could not begin to name everyone, but you know who you are. You've understood when I've missed events, bought me dinner after a rough week, cheered me on through every step of the way.

Dr. Jennifer Hatcher, you have been a tremendous mentor, both personally and professionally. Though I will say a simple thank you here, I believe you know how grateful I am for everything you've done. Ride or die!

To all of the incredible nurses I've known and had the honor of working with, inside and outside of my graduate work, we have developed intense friendships in the most unique of environments. Thank you for sharing your experiences and your lives with me.

TABLE OF CONTENTS

Acknowledgments……..iii

List of Tables……..vi

List of Figures……....vii

Chapter One: Introduction……..……1

Chapter Two: Consequences of Blood Component Transfusion in Patients with Trauma: A Conceptual Model.....6
Introduction……....7
Blood Transfusion and Potential Consequences……..8
Pathophysiology of Trauma……9
Historical Perspective of Transfusion……....13
The Storage Lesion of Cellular Components……....14
Packed Red Blood Cells……14
Platelets……..18
Short-term and Long-term Consequences of Stored Component Transfusion….....21
Evidence-Based Conceptual Model……...25
Conclusion……26

Chapter Three: Association of Blood Component Ratio with Clinical Outcomes in Patients After Trauma and Massive Transfusion: A Systematic Review…..……39
Introduction……41
Methods……..43
Quality of Studies……..43
Results……47
Ratio of Blood Components Administered……47
MT and Outcomes……..49
Discussion……..52
Conclusion……56

Chapter Four: Increased Mortality in Adult Trauma Patients Transfused with Blood Components Compared with Whole Blood……..……75
Introduction……76
Methods……..78
Sample……78
Measures……79
Statistical Analyses……81
Results……82
Characteristics of the Participants……..82
Mortality Predictors.....83
Discussion.....83

Conclusion.....88

Chapter Five: Predictors of Inflammatory Complications in Patients Who Received Component Transfusion After Trauma……..……94
Introduction.....95
Methods.....99
Sample and setting.....100
Measures.....101
Statistical Analyses.....105
Results.....107
Characteristics of the Participants.....107
Prevalence of Complications.....109
Inflammatory Complications Predictors.....109
Time to Complication Development.....110
Discussion.....111
Limitations.....116
Conclusion.....117

Chapter Six: Conclusion…….……....129

References…….……...134

Vita……...179

LIST OF TABLES

Table 2.1a Global Consequences of the Storage Lesion for Packed Red Blood Cells 27

Table 2.1b Global Consequences of the Storage Lesion for Platelets 29

Table 2.2 Thromboelastography Measures and Correlations to Traditional Coagulation Testing 31

Table 2.3 Blood Component Storage 34

Table 3.1 Bias Assessment Instrument 57

Table 3.2 Description of Included Studies (n = 21) 58

Table 4.1 Characteristics of the Participants (n = 1745) 89

Table 4.2 Independent Predictors of Mortality In Adult Trauma Patients (n = 1745) 90

Table 5.1 Characteristics of the Sample (N = 1,538) 118

Table 5.2 Transfusion-Related Characteristics 121

Table 5.3 Inflammatory Complications (N = 1,538) 123

Table 5.4 Predictors of Development of Inflammatory Complications (n = 386) 124

Table 5.5 Predictors of Time to Development of Inflammatory Complications (n = 643) 125

LIST OF FIGURES

Figure 2.1 Effects Leading to Trauma-Induced Coagulopathy 35

Figure 2.2 Thromboelastography Measures 36

Figure 2.3 Conceptual Model of Trauma, Hemorrhage, and Transfusion.....37

Figure 3.1 Article Selection 71

Figure 3.2 FFP:PRBC Mortality by Ratio Group 72

Figure 3.3 PLT:PRBC Mortality by Ratio Group.....73

Figure 4.1 Patient Mortality by Type of Transfusion 91

Figure 4.2 Mean ISS by Transfusion Type.....92

Figure 5.1 Time to development of complications by PRBC:FFP ratio group 126

Figure 5.2 Time to development of complications by PRBC:PLT ratio group.....127

CHAPTER ONE

Introduction

Trauma affects people of all ages, and remains one of the top five leading causes of death in the United States.(1) Uncontrolled hemorrhage is a major cause of death, and is preventable in many situations.(2) The standard of treatment for patients with trauma who experience exsanguination includes infusion of crystalloid fluid and either whole blood or blood components (packed red blood cells [PRBCs], fresh frozen plasma [FFP], and platelets [PLTs]). Due to economic and logistic reasons, transfusion of a combination of blood components rather than whole blood transfusion is the clinical standard of care for patients with trauma in the civilian setting.(3) According to the National Blood Collection and Utilization Report of 2011, 10% of PRBCs and 4% of PLTs were used by emergency and trauma services in United States hospitals.(4) However, less than 1% of all transfusions across the United States were whole blood transfusions, highlighting the lack of whole blood transfusions in the civilian setting.(4)

Transfusion is associated with trauma outcomes indirectly and directly.(5, 6) Indirectly, the volume of blood transfused, and the ratio of blood components are a proxy measure of injury severity; patients with more severe injury typically require more blood volume, and blood components to replace circulatory volume and factors necessary for coagulation.(7) Directly, transfusion is associated with morbidity and mortality by a number of mechanisms associated with the storage lesion, a combination of biological, chemical, and morphological changes in preserved blood components. Broad consequences of the storage lesion in transfused patients include vasoconstriction, systemic inflammation, acidosis, electrolyte imbalances, and decreased tissue

oxygenation.(8-11) The effects of the storage lesion only worsen over time; thus, patients after trauma who receive older stored components, especially in large volume, may experience increased risk of morbidity and mortality.

Consequences of the storage lesion were not recognized until the 1980s, and since that time there has been an ever-growing interest in the subject. Half of all studies published about the PRBC storage lesion were published either during or after 2009.(12) A plethora of evidence exists about mechanisms that produce the cellular morphology and breakdown that occurs with the storage lesion.(13-17) However, the association between the storage lesion and subsequent outcomes has not been fully explored. Recent data provided evidence to support an association between blood volume, ratio of components transfused and increased risk of complications and mortality.(18-22) As a result, current component transfusion practice for all patients is under scrutiny.(17, 23)

In recent years, investigators have initiated studies to test transfusion of fresh whole blood in patients after trauma to reduce morbidity and mortality that resulted from the storage lesion.(23) These studies were spurred by a growing body of evidence that suggested transfusion of blood components in a 1:1:1 fashion, or 1 unit of PRBCs to 1 unit of FFP to 1 unit of PLTs, was beneficial for survival in trauma patients receiving massive transfusion ($\geq$ 10 units PRBCs in 24 hours).(24-26) The rationale for the potential survival benefit was that this combination of transfused components closely mimicked whole blood. However, researchers contended that this benefit may not exist in patients who do not require large volume transfusion.(27)

Thus, trauma patients are exposed to increased risk of complications and mortality when transfused with stored blood components,(28, 29) and evidence to support current

transfusion strategies is lacking. Furthermore, limited research exists which evaluated the relationship between component storage lesion and both short-term and long-term patient outcomes after trauma. Therefore, the purpose of this dissertation was to evaluate the relationship between current trauma transfusion practices and associated outcomes in patients transfused after trauma.

Though the mechanisms of the storage lesion are described in the literature, the relationship between the storage lesion and patient outcomes following trauma and transfusion have not been elucidated. Chapter Two is a paper that presents a conceptual model developed from the current state of the science; the model describes the association of traumatic injury, hemorrhage, and blood component transfusion with short and long term outcomes. In this model, patient outcomes for each of the three components of trauma physiology and management (injury, hemorrhage, and transfusion) are separated into those occurring in the short-term (within 30 days), and those in the long-term (beyond 30 days). Specific emphasis was placed on the consequences of the storage lesion found in erythrocytes and platelets, the combined effects of biological and chemical breakdown that occurs with storage, and the evidence about outcomes associated with the storage lesion.

As the storage lesion impacts each stored unit of erythrocytes and platelets, outcomes for patients who are massively transfused must be considered. Chapter Three contains a systematic review of the literature focused on retrospective, observational investigations performed in civilian and military level I trauma centers, in which outcomes of patients who required massive transfusion after trauma were compared based on the ratio of components transfused. Based on the findings of this systematic

review, transfusion of components in a 1:1:1 ratio demonstrated a significant survival benefit for those patients who required massive transfusion after trauma. However, evidence about the association of transfusion ratio with clinical outcomes (e.g. hospital length of stay or rates of organ failure) was inconclusive.

Chapter Four reported the findings of a secondary analysis of the 2009 National Trauma Data Bank data set. The purpose of this study was to evaluate the relationship between transfusion type (whole blood versus components) and mortality risk in adult patients after major trauma. In this analysis, the type of transfusion was an independent predictor of mortality. Those patients who received components demonstrated a 3-fold risk of mortality likelihood compared with those who received whole blood. This finding is intriguing, though, as transfusion of whole blood in the civilian clinical setting is rare, whereas component transfusion is the standard of care for resuscitation.

Chapter Five reported a secondary analysis of the Inflammation and Host Response to Injury database. The purposes of this analysis were to evaluate: 1) the prevalence of inflammatory complications in blunt trauma patients who received blood transfusions, 2) the relationship between transfusion variables (volume and ratio of components) and the likelihood of an inflammatory complication (e.g. organ failure, pneumonia, septicemia), and 3) the relationship between transfusion variables and time to development of inflammatory complication in adult patients after major trauma. Inflammatory complications included organ failure, septicemia, catheter-related bloodstream infections, urinary tract infections, pneumonia, acute respiratory distress syndrome, and nosocomial infections. Patients included in the analysis received all three components (PRBCs, FFP, and PLTs). The ratios of both PRBC:FFP and PRBC:PLT

were calculated and presented in decimal notation (instead of 3:4, recorded as 0.75 or 0.8). Total transfused volume was converted to units based on average volume of each component.

Findings from this analysis revealed that the vast majority of patients (86%) developed at least one inflammatory complication. Results from a logistic regression determined that the presence of comorbidities and total transfused volume of PRBCs in the first 24 hours of hospitalization were independent predictors of inflammatory complications. There were no significant findings with other transfusion variables. Cox proportional hazard model analysis revealed the total transfused volume of PRBCs and injury severity were independent predictors of time to development of inflammatory complications. There were no differences in time to complications for PRBC:FFP or PRBC:PLT ratio groups.

Chapter Six concludes with an overall summary of findings from the manuscripts in the dissertation and the conclusions developed from these. In this chapter, I present future directions for research and implications for nursing care in this large and important patient population.

CHAPTER TWO

Consequences of Blood Component Transfusion in Patients with Trauma: A Conceptual Model

Synopsis

Blood component transfusion is frequently required in resuscitation of patients with major trauma. Packed red blood cells and platelets experience breakdown and chemical changes during storage (known as the storage lesion) that lead to an inflammatory response once transfused to patients. The pathophysiology associated with the storage lesion, and the relationship between the storage lesion and outcomes of transfused trauma patients are discussed. Outcomes related to trauma, hemorrhage, and component transfusion are presented in a conceptual model, grouped according to those occurring in the short-term (≤ 30 days) and the long-term (> 30 days).

Introduction

Unintentional injury remains one of the leading causes of death for people of all ages in the United States.(1) The most common cause of death for trauma patients within the first 48 hours of injury is exsanguination, accounting for 30-40% of traumatic deaths.(2) Such bleeding, or massive hemorrhage, is defined as either: 1) the loss of blood equal to the circulating volume of the patient within 24 hours; or 2) the loss of half of the circulating volume within three hours.(3) Management of hemorrhage includes fluid replacement and/or transfusion of whole blood or blood components, which include packed red blood cells (PRBCs), fresh frozen plasma (FFP) and platelets (PLTs).

The physiological and clinical consequences of traumatic injury, subsequent hemorrhage, and transfusion include those that occur within 30 days of injury, or short-term, and those occurring beyond the initial 30-day timeframe, or long-term. Short-term consequences of injury, hemorrhage, and subsequent blood transfusion, primarily mortality and major complications during hospitalization, have been described in the trauma literature, especially among trauma patients who received massive transfusion.(4-7) Long-term physiological and psychological consequences of traumatic injury have also been the subject of a multitude of research studies, ranging from rehabilitation (physiological) to post-traumatic stress disorder and depression (psychological) related to major traumatic injury.(8-12) However, the mechanisms connecting trauma, hemorrhage, and transfusion with short- and long-term outcomes have not been thoroughly synthesized.

Blood Transfusion and Potential Consequences

Approximately 1-3% of trauma patients require transfusion of blood components to stabilize their hemodynamic state.(13) In addition, 2% of these patients will require massive transfusion of blood products (10 units or more of PRBCs in a 24 hour period).(3, 14) Transfusion of blood components, in combination with the immediate effects of the trauma, often has multiple detrimental effects for the recipients, resulting in increased risk of morbidity and mortality.

Marik and Corwin (15) performed a systematic review of 45 observational studies of critically ill patients, 10 of which included trauma patients. Using pooled data from these studies, they found PRBC transfusion independently predicted nosocomial infection, with nearly double the likelihood of infection compared to those not transfused (Odds ratio [OR] 1.8, 95% CI 1.5 – 2.2). Additionally, these investigators found that PRBC transfusion independently predicted mortality (OR 1.7, 95% CI 1.4 – 1.9), and development of acute respiratory distress syndrome (OR 2.5, 95% CI 1.6 – 3.3). Other investigators have found similar associations between transfusion of PRBCs and adverse clinical outcomes, such as transfusion-related acute lung injury,(16, 17) acute kidney injury,(18) and thromboembolic events.(19)

Current blood bank practices include rotation of PRBCs near their expiration date to trauma centers more likely to use them before expiration, thus reducing waste.(20) However, predictable and identifiable morphological, biochemical, and functional alterations occur in blood components, known collectively as the storage lesion, which are associated with physiological consequences in those receiving the transfusion (Tables 1).(21, 22) While evidence exists to support associations between patients who received

blood transfusions after trauma and physiological consequences, the underlying pathophysiological mechanisms connecting blood transfusions with these outcomes remain unclear; investigators suggested that these outcomes may be the result of the inflammatory response initiated by transfusion.(23) Thus, the purpose of this paper is to: 1) discuss the relationship between the elements of the storage lesion and consequences of blood transfusions in trauma patients; and 2) to present a conceptual model of the short- and long-term consequences of trauma, hemorrhage, and blood transfusion in this population.

Pathophysiology of Trauma

Traumatic injuries are categorized as blunt or penetrating depending on the mechanism of the insult. Penetrating injuries (i.e. stabbing or gunshot wound), are typically fairly isolated injuries specific to the tissues in the path of the instrument or projectile; these injuries tend to have more severe physiological impact, associated with a five-fold increase in mortality likelihood (OR 5.4, 95% CI 2.4-12.0, $p < 0.01$).(24) Patients with penetrating injuries have a higher likelihood of requiring blood transfusion, particularly massive transfusion.(25-27) In contrast, blunt injuries (i.e. motor vehicle accidents) result in more broadly distributed damage. No matter the mechanism, endothelial disruption and blood loss stimulate a coagulation cascade with the intent of reduction in hemorrhage. In addition, blood is shunted to the brain, heart, and lungs to ensure survival.

Inflammatory cytokines (i.e. interleukin-6 and tumor necrosis factor-alpha) stimulate the recruitment of white blood cells to the site of injury, and initiate a 3-phase response that consists of acute inflammation, repair, and remodeling. Vasoconstriction

occurs immediately after injury to restrict blood loss; this response transitions to vasodilation with increased vascular permeability to increase perfusion, remove foreign bodies, and deliver inflammatory and repair molecules. Vasodilation produces edema, erythema, and in conjunction with release of histamine, bradykinin, and serotonin, causes acute pain.

The initial vasoconstriction after trauma results not only from the injury itself, but also from what is known as the lethal triad, a combination of hypothermia, acidosis, and coagulopathy commonly associated with trauma. Investigators of an 8-year study reported no survivors among those patients who presented to the emergency department with the lethal triad.(28) Thus, understanding the combined effects of the pathophysiology of traumatic injury and the lethal triad is critical to caring for this population.

Hypothermia

Nearly one fifth of traumatic injuries (18%) occurred outside of the home,(29) with trauma patients exposed to the elements for an indefinite period of time; hypothermia may result. Investigators estimated that 13% of trauma patients presented with an admission temperature less than 35°C (95°F),(30) while less than 1.6% had an admission temperature of less than 32°C (89.6°F).(31) Core temperature below 37°C (98.6°F) alter the hemoglobin molecule structure, and produce an increased affinity for oxygen,(32) which results in less oxygen released at the metabolically active cell, and subsequent cellular hypoxia.(32) Persistent hypothermia can result in alterations in cardiac conduction, decreased respiratory rate, and reduced myocardial contractility.(33) Furthermore, investigators found hypothermia independently predicted mortality in

trauma patients, with an increased likelihood of death of nearly 20% (OR 1.19, 95% CI 1.05 – 1.35, p = 0.008).(34) While hypothermia alone may affect the status and outcome of the patient, it is rarely independent of other complications in patients with trauma.

Acidosis

In patients after trauma, clinicians generally attributed acidosis to the production of lactic acid, a metabolite of energy production mechanisms, glycolysis and oxidative phosphorylation.(35) However, trauma can increase the rate of glycolysis to 2 to 3 times more than oxidative phosphorylation due to physiological stress, which results in rapid production of lactate, and subsequent systemic acidosis. In the period immediately following injury, acidosis contributes to development of coagulopathy. Cosgriff and colleagues (36) compared patients who developed coagulopathy with those who did not, and found that a significantly greater proportion of those who developed coagulopathy had a pH < 7.1 compared to those who did not develop coagulopathy (78% vs 32%, p = 0.0004). At a pH below 7.1, patients experienced reduced platelet count and platelet dysfunction, increased fibrinogen breakdown, and impaired plasma protease function.(35) Consequences of persistent acidosis extended beyond the resuscitation period and included decreased cardiac contractility, and reduced renal perfusion.(33) For patients who experienced continued increased lactate levels after traumatic injury, unresolved hemorrhage was the primary suspected mechanism.(33) Investigators examined the association between acidosis and coagulopathy, and found that at a pH of 6.8, thrombus formation time increased by more than 160% compared to thrombus formation times measured at a pH of 7.4.(37) Thus, the pH level and coagulopathic state

directly impact each other, and if unresolved, produce deadly outcomes in patients with trauma.

Coagulopathy

Following activation of the coagulation cascade, patients experience an increase in fibrinolytic activity, and plasminogen activation to balance the degree of coagulation.(38) Patients who experienced widespread tissue damage also developed a systemic coagulation response due to thrombin release.(38, 39) Infusion of crystalloids and blood components during resuscitation may result in a dilutional coagulopathy, especially those patients who received massive transfusion (10 or more units of PRBCs in 24 hours). Unfortunately, coagulopathy after trauma was associated with a 35% increase in likelihood of mortality (Figure 2.).(40) Hypothermia, which is closely associated with coagulopathy, also decreased platelet function.(33) Hypothermia associated with coagulopathy occurs when core temperature was below 33°C (91.4°F).(39) Dirkmann and colleagues (41) reported significant increase in thrombus formation time (approximately 145 seconds) at a pH of 7.26 ± 0.04 with concommitant hypothermia (33°C or 91.4°F), compared to a control (approximately 115 seconds at 36°C or 96.8°F and pH of 7.36, $p < 0.05$) which highlights the interplay of the lethal triad. Investigators concluded that for patients with devastating injury, this combination required aggressive resuscitation measures to correct coagulopathy and stop ongoing hemorrhage.(35, 42)

Assessment of coagulation

Standard measures of coagulation include prothrombin time (PT), activated partial thromboplastin time (aPTT), and the International Normalized Ratio (INR). While these measures reflect the ability to coagulate and initiate hemostasis, the logistics of

measurement require a prolonged waiting period for results (typically around 30 minutes or more), which can delay treatment. Patients after trauma present a unique challenge in the immediacy of their coagulation needs; therefore, clinicians and researchers have begun using thromboelastography to guide resuscitation.

Thromboelastography (TEG) is a measure of the viscoelastic changes that occur during coagulation and lysis of whole blood (Table 2, Figure 3.).(38, 43) TEG measures the time for thrombus generation, the strength of the thrombus, as well as lysis of the thrombus.(43, 44) Investigators found strong correlations between TEG values and traditional coagulation values in patients after trauma (e.g. PT, aPTT), which provided evidence to support the use of TEG for resuscitation.(45) TEG results are completed within 20 minutes, which provides clinicians with data upon which to base resuscitation.(45, 46) The primary drawbacks to TEG are the cost of the testing, and the time required (approximately two days) for personnel training in TEG use and interpretation.(46, 47) Overall, evidence to support the use of TEG in the trauma setting as a guide for resuscitation continues to grow.(48-50)

Historical Perspective of Transfusion

Transfusion originally involved transfusion of whole blood. With World War II, the United States military found it necessary to preserve and send blood to the front lines for resuscitation of injured soldiers.(51) Thus, scientists developed procedures to preserve blood components like albumin and plasma proteins for transfusion. In the 1950s, the development of the cell separator led to the transfusion of blood components like packed red blood cells and fresh frozen plasma, which currently remains the standard in civilian

practice. Discussion of transfusion of stored components necessitates understanding of the associated storage lesion.

The Storage Lesion of Cellular Components

Changes associated with the storage lesion include: 1) cellular and morphological changes, 2) oxygenation and energy changes, 3) biochemical changes, 4) release of microparticles, and 5) increase in inflammatory mediators. The storage lesion develops in a predictable fashion over time, such that the longer the component is stored, to the greater degree of disruption to cellular integrity is detected (Table 3).

Packed Red Blood Cells

Cellular and Morphological Changes

During storage, PRBCs experience cell shape and membrane integrity alterations, with change from the normal smooth, flexible disc to spherical cells with sharp protrusions, called spheroechinocytes.(52, 53) Karon and colleagues (54) found that these morphological changes occurred rapidly, and early in the storage period, with 9.5% of cells deemed abnormal (i.e. spheroechinocytes) by day seven of storage. Due to their new shape and lack of flexibility, spheroechinocytes are unable to pass through the microvasculature, because the protrusions are more likely to cause the cells to adhere to the endothelium.(55, 56) Anniss and Sparrow (57) found that the number of adherent red blood cells increased significantly from 69 ± 10 cells per mm^2 on the first day of storage to 128 ± 11 cells per mm^2 on storage day 42 ($p < 0.05$).(57) Consequently, transfused PRBCs have significantly reduced flow through the microcirculation, with less oxygen delivered to metabolically active cells; these abnormal erythrocytes also obstruct the

microcirculation, and may result in cellular ischemia, and tissue and organ dysfunction.(55, 58)

Oxygenation and Energy Changes

During storage, biochemical changes result in a reduction in 2,3-diphosphoglycerate (2,3-DPG), a byproduct of glycolysis in the red blood cell.(21) A reduction in 2,3-DPG produces a left shift of the oxyhemoglobin dissociation curve; thus, the beta chain of the hemoglobin molecule will have greater affinity for the oxygen molecule, and will not release oxygen at the cell. Almac and Ince reported that the 2,3-DPG concentration fell below detectable levels within two weeks of preservation.(59) The increased oxygen affinity in transfused PRBCs will continue until the recipient produces adequate 2,3-DPG levels. However, 2,3-DPG levels in transfused patients return to normal within the first few days after transfusion.(60, 61) Stan and colleagues found that 2,3-DPG levels reached more than 60% of normal value (measured at 72 hours) within 24 hours of transfusion completion.(60) However, for patients who received massive transfusion, replacement of their entire blood volume, lower levels of 2,3-DPG transfused in large volumes of stored components may significantly reduce cellular oxygen concentration for several hours after transfusion completion.

Erythrocyte metabolism continues after initiation of the storage and preservation process, as the supernatant, or the fluid in which the cells are stored, contains glucose. In the absence of oxygen, erythrocytes convert to anaerobic metabolism of glucose to produce adenosine triphosphate, or ATP.(62) Investigators recently reported a 1 to 2-fold increase in ATP levels during the first two weeks of storage for PRBCs, with levels falling below their initial levels by the end of the 42-day storage period.(63) This initial

rise in ATP levels was attributed to release of ATP in response to a hypoxic state, where ATP acted as a vasodilator in vivo.(64) Krager and colleagues examined 40 units of leukocyte-depleted whole blood, and described a change in ATP levels from an average of 3.5 $\pm$ 0.4 μmol/g of hemoglobin (Hb) on day 1 to an average of 2.3 $\pm$ 0.4 μmol/g Hb on day 42.(65) With continued anaerobic metabolism and limited stores of glucose available, the finite amount of ATP found in the stored components gradually decreased during storage. ATP depletion was associated with cellular alterations, as the ATP pump within the cell membrane failed, and resulted in sodium accumulation within the cell; this lead to stiffened cellular structure due to swelling, and loss of phospholipid membrane through formation of microvesicles (small pieces of the membrane that break off).(66) The combination of altered cellular chemistry and structure resulted in decreased oxygenation ability through increased oxygen affinity and inability to travel through microvasculature. Extended tissue oxygen deprivation may result in organ damage and/or dysfunction, especially to those tissues most sensitive to hypoxia, myocardial and cerebral tissues, for example.(67)

Biochemical Changes

Anaerobic metabolism results in by-products, that include lactic acid, and excess hydrogen ions from breakdown of fatty acids for additional energy, both ultimately lead to a reduced intracellular pH level following persistent hypoxia.(67) With decreased ATP available for essential functions, like the sodium-potassium pump, cells accumulate sodium as the pump fails; this results in cellular edema, weakening of the cell membrane, and cellular internal structures, such as the mitochondria.(67) Extracellular potassium concentrations also increase with failure of the sodium-potassium pump. Investigators

found significant increases in plasma potassium levels during storage, from 5.16 ± 1.2 mmol/l on day 0 to 35.1 ± 4.6 mmol/l on day 28 ($p < 0.005$).(68) Two developments characterize irreversible cellular damage, cessation of mitochondrial function, and the loss of membrane function.(67) Irreversible damage such as this occurs in approximately 50% of PRBCs by day 21 of storage.(53) Once transfused, damaged red blood cells may succumb to apoptosis, or be removed from the circulation similar to normal processing of old red blood cells, thereby decreasing the overall effectiveness of the transfused unit.

Release of Microparticles

After the cell membrane is no longer intact, intracellular fluid and cellular contents leak into the extracellular space. Several substances escape from cells with cellular structure breakdown; these include microvesicles containing plasma membrane components, lipids, free hemoglobin, free iron, and cytokines. Chaudhary and Katharia (68) found a significant increase in plasma hemoglobin during storage (day 0 0.017 ± 0.01 g/dL, day 28 0.077 ± 0.06 g/dL, $p < 0.005$). Release of microvesicles is associated with decreased endothelial-derived nitric oxide, which is a potent vasodilator and antioxidant,(52) and induction of a hypercoagulable state.(69) Free hemoglobin and free iron contributed to oxidative stress in the cells, with subsequent free radical production, which may initiate tissue damage once transfused.(52) Longer storage time is associated with greater release of microvesicles.(69) Thus, given that trauma centers receive older stored PRBCs, the concentration of microvesicles is likely substantively elevated.

Increase in Inflammatory Mediators

The storage lesion is also associated with an increased release of inflammatory mediators. Kor and colleagues (58) found cytokine and other proinflammatory mediator

concentrations rose throughout the duration of storage in 22 units of leukocyte-reduced PRBCs. Their findings suggested that despite the removal of white blood cells, some proinflammatory molecules remained in stored PRBCs. Ubiquitin, a protein found in all eukaryotic cells, related to inflammation through inhibition of tumor necrosis factor-alpha, is also released with the breakdown of the cell membrane.(70, 71) Patel, Proctor and Majetschak (71) found a significant increase in ubiquitin levels during storage, ranging from 113 ± 33 ng/mL on day 0 to 2170 ± 268 ng/mL on day 42 ($p < 0.001$). Thus, through transfusion of PRBCs, patients experience an inflammatory reaction and subsequent adverse outcomes.

Platelets

Cellular and Morphological Changes

Although similarities exist between PRBC and PLT storage lesion effects, our current understanding about the PLT storage lesion lacks in comparison to that of PRBCs. Storage of PLTs stimulated a process that mimicked PLT activation in vivo,(72) which ultimately resulted in membrane breakdown and alteration of the cell shape, changing the cell from a smooth, plate-shaped structure to a rounded cell with spiny protrusions.(73) Through PLT activation, membrane breakdown, and exposure of phosphatidylserine (a phospholipid found on the inner portion of the platelet cell membrane), PLTs may develop procoagulant properties, whereby they become capable of thrombin generation, and premature cell death while in storage or soon after transfusion.(74) Investigators reported a minimal decrease in the efficacy of transfused PLTs, but determined that structurally altered PLTs are removed from circulation more

rapidly than non-altered PLTs, due to stimulation of the apoptosis cell death pathway via structural change.(73)

The most effective method of PLT storage remains controversial, with the primary challenge centered on the balance between maintenance of cellular integrity and reduction in risk of bacterial contamination. Current standards include storage of PLTs at room temperature with continuous agitation for 5 days.(73, 75) Previous standards, however, included refrigeration of PLTs during storage, which was intended to decrease likelihood of bacterial contamination.(73) Change in these standards was based on research findings that determined cold storage temperature was associated with premature PLT removal from the circulation via the liver; thus, cold storage reduced PLT circulation time.(76) Researchers evaluated PLTs stored at 4°C (39.2°F) for at least 48 hours prior to transfusion in mice, and found that approximately 50% of the transfused PLTs were cleared from circulation within 2 hours.(77) Recently, investigators suggested that refrigerated PLTs might be more beneficial to trauma patients, as they are somewhat activated prior to transfusion, and therefore contributed to hemostasis more rapidly.(76) Thus, while PLTs experienced morphological changes and activation during storage, they remained effective, albeit for a shorter period of time.

Oxygenation and Energy Changes

Like PRBCs, anaerobic metabolism during PLT storage often leads to depletion of ATP, and consequently, breakdown of the plasma membrane and leak of the intracellular contents into the extracellular fluid.(74) Investigators described a statistically significant loss of PLTs when ATP levels fell below 2 μml/10^{11} PLTs ($p < 0.001$).(78) PLTs survived for a maximum of 9-10 days in vivo before apoptotic cell death

(programmed death). However, an alternative form of cell death may occur. Investigators suggested that stored PLTs experienced increased necrotic cell death, which was associated with ATP depletion during storage. Necrotic cell death included destruction of the plasma membrane, and edema of the cell and cell organelles, which subsequently lead to cell lysis.(74) No matter the cause of cell death during storage, the result is the same, breakdown of the cell, and activation of a procoagulant state.

Biochemical Changes

PLT necrosis during storage resulted from initiation of one of many death activation pathways (e.g. hypoxia), each culminated with the cessation of mitochondrial function and ATP depletion.(74) A major consequence of this, the increased production of reactive oxygen species, is associated with the destruction of organelles, the plasma membrane, and ultimately the cytoskeleton.(74) PLTs also reverted to anaerobic metabolism in the absence of glucose, with subsequent accumulation of lactic acid, hydrogen ions, and consequent decreased pH. Depletion of ATP also resulted in failure of ATP-dependent processes, which led to electrolyte imbalances in PLTs similar to those in PRBCs.(74) Thus, multiple sources of cellular injury are described during PLT storage.

Release of Microparticles

PLT activation includes expression of membrane receptors, which triggered release of microparticles.(79) Investigators suggested that these microparticles have procoagulant properties, which makes them beneficial to recipients, but may also stimulate an immune response.(79) In addition, PLTs contain three types of granules with proinflammatory properties; these include alpha granules (primarily proteins, i.e. P-selectin), dense granules (contain small molecules, i.e. serotonin or ATP), and lysosomal

granules (contain degradative enzymes).(79) The mechanisms by which microparticles trigger inflammation have yet to be fully elucidated.

Increase in Inflammatory Mediators

Storage may stimulate PLT activation, which resulted in necrotic cell death; these effects worsen over storage time. PLT activation also triggered development and release of inflammatory mediators (thromboxane and prostaglandins), which stimulated an inflammatory response in the recipient.(79) Investigators also suggested that transfusion of PLTs after necrotic cell death exacerbated recipient inflammation.(74) Furthermore, PLT transfusion commonly stimulated inflammation, with receipt of "foreign" PLTs from a donor.(79) Inaba and colleagues reported that trauma patients who received apheresis PLTs stored for 4 days and 5 days were approximately 20% more likely to experience development of complications when compared with those who received apheresis PLTs stored ≤ 3 days ($p < 0.001$).(80) However, investigators currently lack a full understanding of the mechanisms responsible for this relationship.

Short-term and Long-term Consequences of Stored Component Transfusion

Reperfusion Injury

One of the more immediate consequences of transfusion of stored blood components is reperfusion injury. Reperfusion injury occurs when tissues deprived of adequate circulation subsequently receive oxygen and nutrients with restored circulation. In highly oxygen-dependent tissues such as the brain, damage can occur after mere seconds or minutes of oxygen deprivation.(81) Under normal circumstances, the body both produces and sufficiently processes reactive oxygen species (ROS) with the use of antioxidants available to the tissues. With reperfusion injury, when oxygen is restored to

hypoxic tissues, the result is an over-production of ROS that the body is ill-equipped to manage due to a limited supply of antioxidants.(81) In addition, nitric oxide (a vasodilator produced by endothelial cells in normal conditions, but produced by smooth muscle cells in a state of hypoxia) levels decreased, as the cells are now provided with adequate oxygen and circulation is restored.(82)

Once released from the mitochondria, these ROS cause additional irreversible cellular damage in addition to the initial hypoxic insult.(81) When reperfusion injury occurs, it stimulates an inflammatory response, where neutrophils are recruited to the site of injury.(83) In recent years, the focus of reperfusion injury and its associated outcomes has included areas such as cardiac arrest and resuscitation, hypoxic-ischemic brain injury, or stroke.(81) Kunimatsu and colleagues (84) reported that ROS development was greatest in the first 15 minutes following reperfusion in patients with "global brain ischemia". In spite of our current understanding about reperfusion injury, little research exists about the consequences of reperfusion injury after trauma and subsequent transfusion.

Vasoconstriction

Traumatic injury induces vasoconstriction to shunt blood to vital organs, and reduce blood loss at the site of injury. Furthermore, traumatic injury associated with environmental exposure induces vasoconstriction to preserve body heat. To restore blood volume, blood or blood components and intravenous fluids infusion is the current standard for resuscitation. Blood and fluid warmers increase the temperature of infused fluids; however, these warmers may not increase the temperature of infused fluids to that of body temperature. Thus, patients hypothermic due to exposure are infused with cold or

cool fluids in typically large volumes, which further stimulates vasoconstriction, and subsequent reduction of circulation to metabolically active tissues. In addition, patients after trauma are likely to receive older stored blood components with greater acidity, which will exacerbate vasoconstriction.

Inflammation

One of the consequences of the storage lesion is morphological alteration of the shape of both PRBCs and PLTs, and the release of microparticles (MPs) from these cells; these MPs stimulate an inflammatory response. PLTs play a significant role in the immune response, thus transfusion of PLTs with foreign antibodies also generates an inflammatory response.(79) Such response can lead to a transfusion-related reaction; signs normally associated with such reactions (i.e. fever, rash, erythema) occur within minutes of transfusion. In fact, PLT transfusions induced approximately three times more adverse effects than PRBC transfusions; PLT-associated adverse events occurred in one out of every 1,030 PLT transfusions.(85)

MPs consist of small pieces of plasma membrane expelled by cells of all types when stimulated by a stressor or trigger (e.g. initiation of cell death pathways); once the trigger initiates this process, the number of microparticles released only increased.(86) Microparticles are shed from cells on a regular basis, and are capable of expression of surface markers of their cell origin.(86) In other words, these MPs released from PLTs are able to impact inflammatory reactions, among other cellular activities, just as PLTs do.(86, 87) Investigators suggested that microparticles from PLTs suppressed immune response after transfusion; thus further contributing to the development of

complications.(88) The extent of the relationship between microparticles and clinical complications after transfusion has yet to be fully elucidated.

Impaired immune reaction

The consequences of traumatic injury and resuscitation include hypothermia, acidosis, and decreased metabolic activity, with greater energy expenditure. Initially, an inflammatory response is stimulated; however, after this initial response there is initiation of an anti-inflammatory state as healing begins.(83) Xiao and colleagues investigated leukocyte gene expression in patients after trauma, measured immediately following injury, first within 12 hours of injury, then on days 1, 4, 7, 14, 21, and 28.(89) They found that patients with severe trauma experienced a change in more than 80% of the leukocyte gene expression within 28 days of injury. In addition, the most notable change in gene expression occurred within the first 12 hours following injury. Of the genes in which expression increased the most, 80% were involved with inflammation or innate immunity; of those that were notably inhibited, 90% were associated with antigen presentation and activation of T cells. The investigators also reported that over 50% of the genes examined had not returned to normal after 28 days. In some cases, these continued anti-inflammatory responses, when combined with endothelial damage and other factors such as comorbidities, lead to complications like acute respiratory distress syndrome or multiple organ failure.(83) Consequently, patients experienced an increased possibility of infection via open wound, through invasive procedure, or development of inflammatory complications during hospitalization. The prolonged effects of this impaired immune reaction remain unknown.

Clinical complications

In a recent meta-analysis, investigators evaluated studies comparing outcomes of patients based on transfusion of aged stored blood, defined as anywhere from 9 to 42 days in storage.(90) They found that in seven of 21 studies, receipt of older PRBC transfusions was associated with development of complications during hospitalization, that included pneumonia, deep vein thrombosis, acute respiratory distress syndrome, multiple organ dysfunction syndrome, renal failure, sepsis, acute renal failure, and sepsis. The overall estimate of increased risk included increase in risk of multiple organ dysfunction syndrome (OR 2.26, 95% CI 1.56-3.25) and pneumonia (OR 1.17, 95% CI 1.08-1.27). The relationship between blood component transfusion and complications, and the mechanisms by which the storage lesion affects these outcomes remains unclear. However, evidence such as that presented here suggested that detrimental effects are associated with transfusion of older components. With current blood bank practices, older units of blood components are sent to trauma centers; thus, patients transfused following major trauma may experience a greater risk of complications. Few investigators have examined long-term effects of transfusion. Possibilities for future research of such outcomes include, but are not limited to chronic organ dysfunction, cognitive dysfunction, physical or mental disability, awareness of disability, hospital readmission, rehabilitation requirements, and return to baseline status.

Evidence-Based Conceptual Model

Consequences of physical trauma and hemorrhage have been thoroughly examined in the healthcare literature. Currently, knowledge about transfusion after trauma and the storage lesion focuses primarily on short-term, clinical complications

likely to develop during hospitalization. For those patients who survive past their hospitalization, however, there is a lack of knowledge and evidence about the potential long-term consequences of the storage lesion and transfusion following traumatic injury. Thus, the conceptual model presented here (Figure 1.) was developed as a representation of the current state of knowledge about the relationship between traumatic injury, hemorrhage, and transfusion of blood components with both short- and long-term consequences. Areas on the top half of the model denote short-term consequences, and those on the bottom denote the long-term consequences. As indicated by the area at the bottom, right-hand corner of the figure, the opportunities for exploration of long-term transfusion consequences are essentially unlimited and untouched.

Conclusion

Trauma affects a significant portion of the population annually, with severely injured patients in need of transfusion of blood components and fluid for resuscitation. Current standards of blood component preservation support extended storage periods, which are associated with deleterious effects on the cells, the storage lesion. The short- and long-term consequences of trauma and short-term consequences of hemorrhage have been well described; further research is required to determine the extent of long-term consequences of hemorrhage, paired with the consequences of stored component transfusion.

Table 2.1a Global Consequences of the Storage Lesion for Packed Red Blood Cells

Storage Lesion Alterations	Storage Lesion Effects	Physiological Consequences After Transfusion
Cellular and Morphological Changes	• Irreversible change from smooth, easily deformable discs to spheroechinocytes (less flexible, spherical cells with protrusions) • Development of microvesicles with procoagulant properties	• Difficulty moving through smaller blood vessels • Unable to oxygenate tissues adequately • Reperfusion injury due to release of free radicals • Inflammation due to spheroechinocytes adhering blood vessels
Oxygenation and Energy Changes	• Reduction in 2,3-DPG • Impaired ability to carry/deliver oxygen • Decrease in ATP	• Left shift in oxyhemoglobin dissociation curve • Impaired circulation and inadequate tissue oxygenation • Failure of ATP pump; cellular swelling

Table 2.1a, Cont.

Biochemical Changes	• Decrease in ATP • Switch from aerobic metabolism to anaerobic metabolism	• Failure of ATP pump; electrolyte imbalance of transfused supernatant (increased potassium); increased lactate • Excess hydrogen ions, build up of lactic acid, decrease in pH • Irreversible cellular damage
Release of Microparticles	• Breakdown of phospholipid membrane; microparticles released into supernatant	• Increase in free hemoglobin, free iron, cytokines, plasma membrane • Vasoconstriction; induction of hypercoagulable state, tissue damage, and inflammation
Increase in Inflammatory Mediators	• Due to breakdown of cellular structure and leaking of cellular contents	• Increase in ubiquitin and cytokine levels throughout storage • Induction of inflammatory reaction

2,3-DPG = 2,3-diphosphoglycerate; ATP = adenosinetriphosphate

Table 2.1b Global Consequences of the Storage Lesion for Platelets

Storage Lesion Alterations	Storage Lesion Effects	Physiological Consequences After Transfusion
Cellular and Morphological Changes	• Irreversible change from smooth, easily deformable plate-shaped cells to spheroechinocytes (less flexible, spherical cells with protrusions) • Platelet activation • Development of microvesicles	• Difficulty moving through smaller blood vessels • Membrane breakdown; exposure of phosphatidylserine; potentially early cell death • Development of procoagulant properties
Oxygenation and Energy Changes	• Switch from aerobic metabolism to anaerobic metabolism • Decrease in ATP	• Breakdown of plasma membrane, leaking of intracellular contents • Potential cellular death due to membrane breakdown • Build up of lactic acid, excess hydrogen ions, drop in pH
Biochemical Changes	• Decrease in ATP	• Failure of ATP pump; electrolyte imbalance; cellular swelling

Table 2.1b, Cont.

Release of Microparticles	• Expression of membrane receptors; phospholipid membrane breakdown	• Release of microparticles with procoagulant and pro-inflammatory properties
Increase in Inflammatory Mediators	• Platelet activation; breakdown in cellular structure	• Development and release of inflammatory mediators

ATP = adenosinetriphosphate

Table 2.2 Thromboelastography Measures and Correlations to Traditional Coagulation Testing (44,45)

TEG Value	Measures	Correlation to Traditional Coagulation Tests	Normal Range	Transfusion Recommendation	P Value
R time*	Coagulation factors (time to initial fibrin formation)	PT aPTT PLT	5-10 min	> 10 min: FFP, cryoprecipitate	PT: < 0.001 aPTT: < 0.001 PLT: 0.12
K time**	Fibrinogen, platelet number (time for thrombus to reach 20 mm thrombus strength)	PT aPTT PLT	1-3 min	> 3 min: FFP, cryoprecipitate	PT: < 0.001 aPTT: < 0.001 PLT: 0.02

Table 2.2, Cont.

α angle	Fibrinogen, platelet number (angle from baseline to slope of tracing – indicates thrombus formation)	PT aPTT PLT	53° – 72°	< 53°: platelets with or without cryoprecipitate	PT: < 0.001 aPTT: < 0.001 PLT: < 0.001
MA	Platelet function (maximum amplitude of tracing)	PT aPTT PLT	50 – 70 mm	< 50 mm: platelets	PT: < 0.001 aPTT: < 0.001 PLT: < 0.001
G value	Coagulation cascade	PT aPTT PLT	5.3 – 12.4 dynes/cm^2	N/A	PT: 0.005 aPTT: -0.19 PLT: 0.03

Table 2.2, Cont.

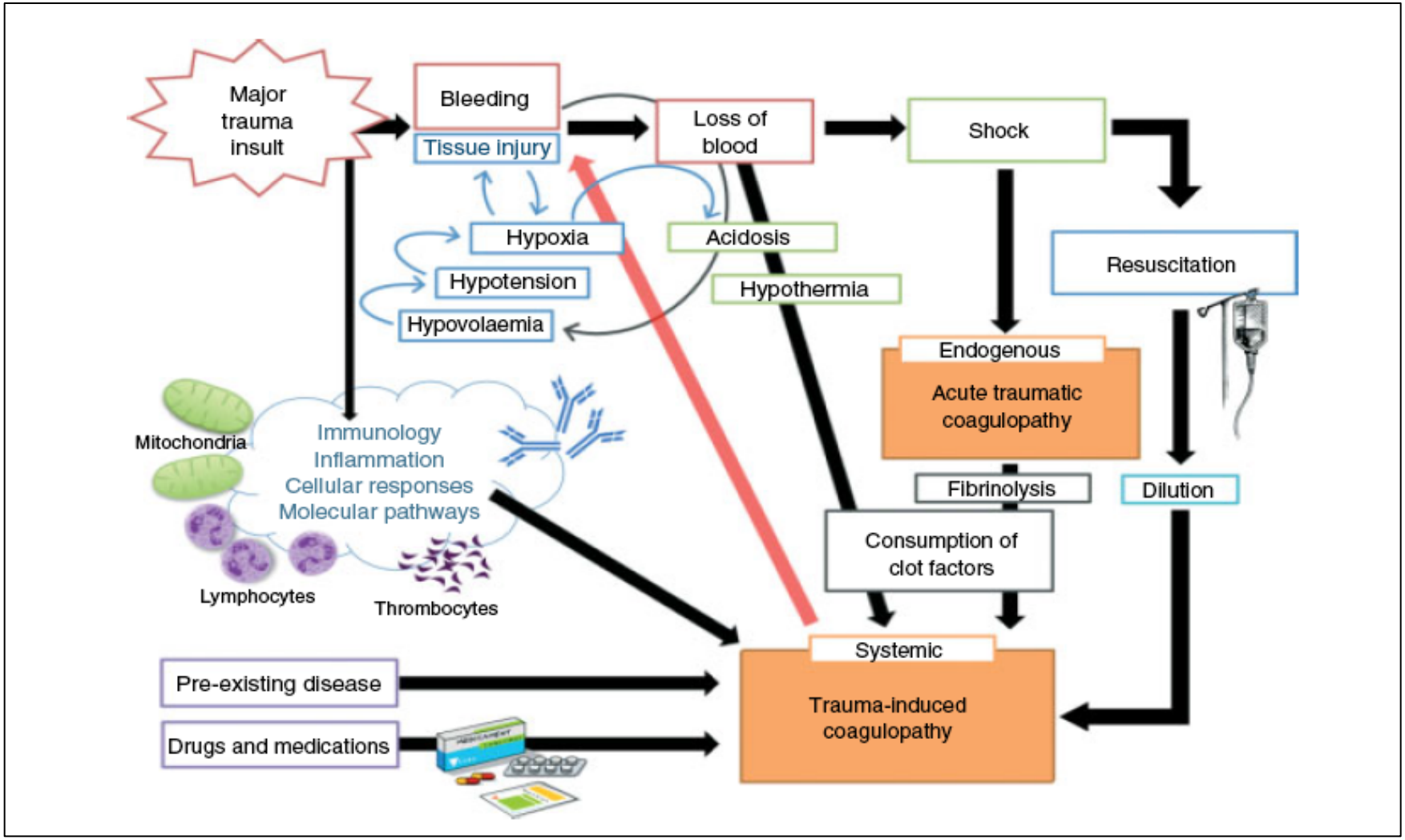
LY 30 ††	Fibrinolysis (thrombus lysis at 30 minutes following MA)	N/A	0-3%	> 3%: tranexamic acid	N/A

R time = Reaction time; K time = Kinetic time; MA = Maximum amplitude; G value = calculated value of clot strength; LY30 = clot lysis at 30 minutes; PT = prothrombin time; aPTT = activated partial thromboplastin time; PLT = platelet count

Table 2.3 Blood Component Storage (91)

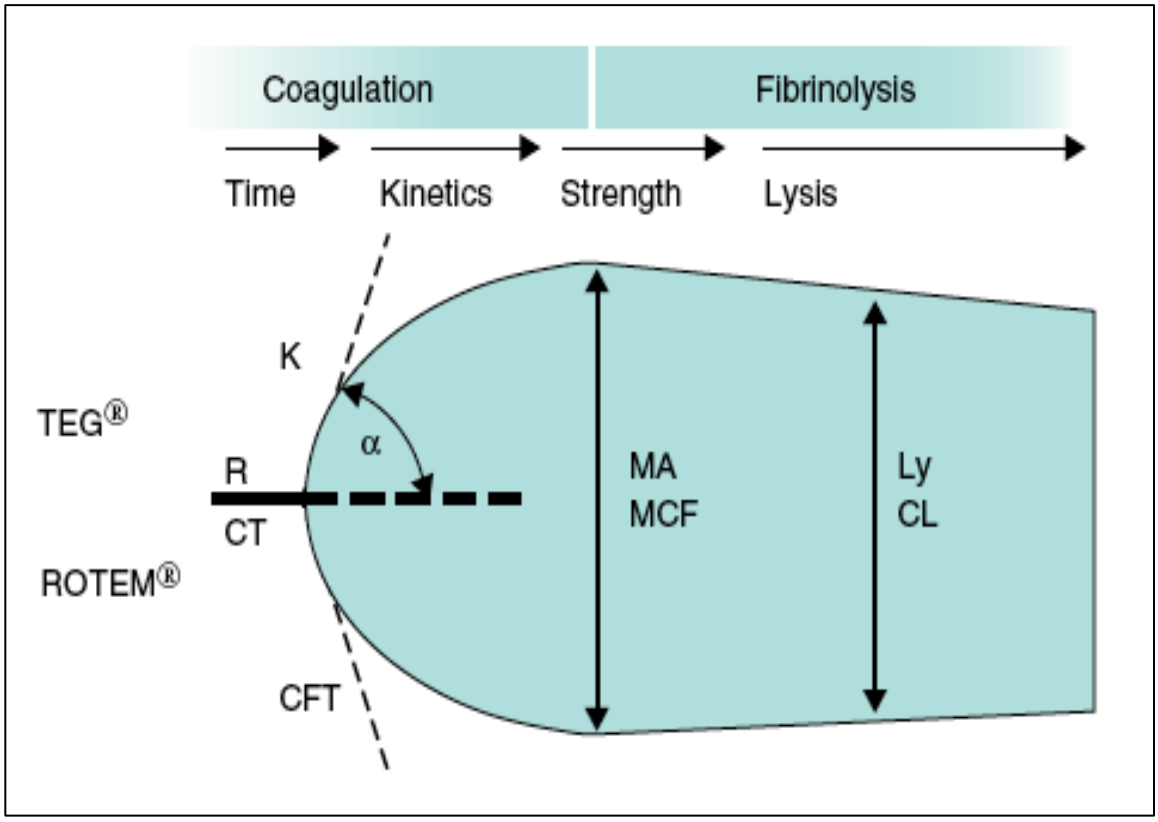
Component	Storage Method	Maximum Storage Time	Preservatives	Additives
Packed red blood cells	Refrigerated	42 days	Anticoagulant – some combination of citrate, phosphate, dextrose, and/or adenine	One of the following: Adsol®, Nutricel®, Optisol®, SOLX®
Fresh frozen plasma	Frozen	1 year; if thawed, must be used immediately	Anticoagulant	N/A
Platelets	Room temperature, with constant agitation	5 days	Anticoagulant (apheresis only)	One of the following: InterSol®, Isoplate™

Figure 2.1 Effects Leading to Trauma-Induced Coagulopathy



Reproduced with permission. Clinical and cellular effects of hypothermia, acidosis and coagulopathy in major injury. Thorsen et al. *British Journal of Surgery, 98*(3). ©2011 British Journal of Surgery Society Ltd.

Figure 2.2 Thromboelastography Measures



Reproduced with permission. Clinical and cellular effects of hypothermia, acidosis and coagulopathy in major injury. Thorsen et al. *British Journal of Surgery, 98*(3).

Figure 2.3 Conceptual Model of Trauma, Hemorrhage, and Transfusion

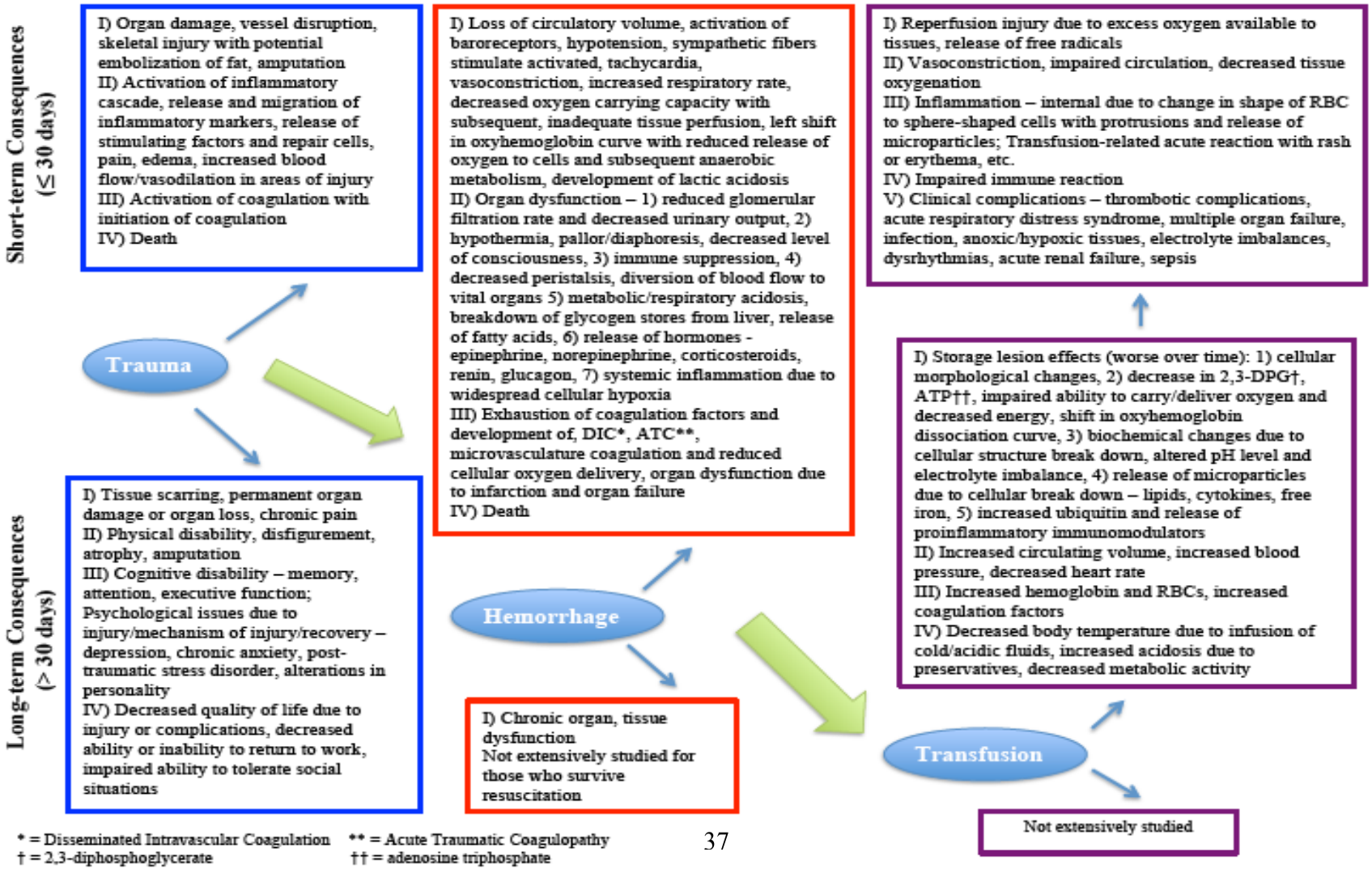


Figure Legend

Figure 2.1 Effects Leading to Trauma-Induced Coagulopathy

Figure 2.2 Thromboelastography Measures

Figure 2.3 Conceptual Model of Trauma, Hemorrhage, and Transfusion

CHAPTER THREE

Association of Blood Component Ratio with Clinical Outcomes in Patients After Trauma and Massive Transfusion: A Systematic Review

Synopsis

Objective: To systematically review studies that compared clinical outcomes of patients after trauma that required massive transfusion (10 or more units of packed red blood cells in 24 hours) based on the ratio of blood components received.

Summary Background Data: Hemorrhage is the primary preventable cause of death in trauma patients. Volume resuscitation includes infusion of crystalloids and transfusion of blood components (packed red blood cells, platelets, fresh frozen plasma). However, the most effective ratio of massively transfused components in trauma patients has yet to be determined.

Methods: PubMed, CINAHL, and MedLine (Ovid) were searched for studies published between 2007 and 2014. We systematically reviewed selected studies using an adapted 9-item instrument to assess bias in observational studies. Patient outcomes were determined and compared based on ratio of components transfused.

Results: Twenty-one studies were included in the analysis; only two were prospective, the remaining 19 were retrospective. The average bias score for the studies was 2.86 ± 1.39 out of a maximum of 16, indicating low risk for bias among the investigations. The most common sources of potential bias were lack of data about primary outcomes and adverse events. Transfusion of components in a 1:1:1 fashion was associated with a

survival benefit during hospitalization, but findings were equivocal for clinical outcomes such as ventilator days and length of stay.

Conclusions: Transfusion of blood components in a 1:1:1 ratio is associated with improved survival in patients with trauma who required massive transfusion. The effect of component ratio on other clinical outcomes requires further investigation.

Introduction

Trauma is the fifth leading cause of death for people of all ages;(1) deaths attributed to unintentional traumatic injury totaled more than 187,000 individuals in the United States in 2011.(2) Trauma hemorrhage was responsible for approximately 50% of all trauma mortality in 2013,(3) and half of trauma deaths that occurred within the first 24 hours after injury.(4, 5) Traumatic hemorrhage was often complicated by coagulopathy, which subsequently worsened the hemorrhage. Recent evaluation of a large trauma database revealed that based on international normalized ratio (INR) and partial thromboplastin time (PTT) values, 42% (INR) and 21% (PTT) of trauma patients, respectively, were coagulopathic on arrival to the emergency department (ED).(6) Coagulopathy has been associated with a 4-fold increase in risk of mortality in trauma patients.(7)

In 2014, investigators reported findings from the Clinical Randomization of an Antifibrinolytic in Significant Haemorrhage-2 (CRASH-2) clinical trial, which included outcomes of more than 27,000 trauma patients from 40 countries; they found that approximately 50% of these trauma patients required transfusion of at least one unit of packed red blood cells (PRBCs) for stabilization.(8) In 2011, trauma and emergency patients received 10.2% of all PRBC transfusions, and 4.4% of all platelet (PLT) transfusions given in American hospitals.(9) According to a recent study of multiple trauma centers, investigators found that approximately 14% of trauma patients who required transfusion received massive transfusion (MT) of blood components.(10) MT has been typically defined as transfusion of 10 or more units of PRBCs in a 24-hour period.(3, 10) Those patients who required MT after trauma hemorrhage were eight times

more likely to develop infection during hospitalization, compared to similarly injured patients who were not transfused (OR 7.97, 95% CI 2.3-27.5, $p < 0.001$).(11) Trauma patients who received MT also required an average of 5 days longer in the intensive care unit and a 14-day longer hospital length of stay, when compared with those similarly injured, but not transfused.(11) Thus, patients who required MT after trauma are a highly vulnerable population.

Facilities with Level I trauma centers may use a MT protocol to guide management of severe hemorrhage. MT protocols commonly include policies to facilitate the rapid release and delivery of blood components to the patient. Protocols specify those who can activate the protocol, volume of blood components and ratio of components to be released and administered, required laboratory testing prior to and during implementation of the protocol, and outcomes to be evaluated.(12) However, not all trauma centers have these. In 2010, investigators found that although 85% of trauma centers had a MT protocol in place, the majority (65%) of these had been in place for less than five years.(13) Unfortunately, of those facilities with protocols, there was a serious lack of consistency in the number of units, types of components released, and ratio of components to be administered with activation of the protocol. To date, evidence-based practices for MT have not been comprehensively evaluated and synthesized. Thus, the aim of this review was to systematically analyze studies where outcomes were compared based on ratios of blood components administered to adult trauma patients who required MT at Level I or major trauma centers in both military and civilian institutions.

Methods

We searched PubMed, CINAHL, and MedLine (Ovid) using the key words blood, massive transfusion, emergency, trauma, and ratio, and included studies that: 1) were published in English between 2007 and 2014; 2) were performed at military or civilian Level I or major trauma centers; 3) focused solely on adult trauma patients who received massive transfusion as defined by the investigator; and 4) compared outcomes of groups based on the ratio of blood components administered. The initial search resulted in a total of 664 articles. Examination of title and abstract for relevance, and exclusion of duplicates resulted in 33 articles for inclusion. Hand search and further evaluation of the studies for inclusion limited the review to 21 published studies (Figure 1).

We evaluated the risk of bias in the reviewed studies using a 9-item instrument based on Viswanathan and Berkman,(14) who identified indicators of bias in observational studies (Table 1.). Each published study was evaluated independently by two reviewers for 9 items that evaluated for risk of bias; four items were scored 0 to 1; three items were scored 0 to 2; two items were scored 0 to 3. Item scores were summed for a total score, which ranged from 0 to 16; higher scores indicated greater risk for bias. Scores from the two independent reviewers agreed in 98% of cases. Discrepancies in scoring were discussed until 100% agreement was reached.

Quality of Studies

This review included 21 studies (Table 2.). Only two of the included investigations (15, 16) were prospective; all others were retrospective medical record or database reviews.(17-35) Seven studies (32%) used multicenter data for their analyses.(16, 20, 22, 27, 30, 32, 33) The majority of studies were performed in the United

States (62%)(15, 16, 18-21, 23, 25-27, 30, 31, 33) and in civilian trauma centers (57%).(15, 16, 18-21, 23, 25-27, 30, 31) Studies performed outside of the United States included those from Germany;(22, 32) Australia,(28) and Japan.(35) Three studies were performed in Combat Support Hospitals in Iraq;(17, 24, 34) the investigators of a fourth military study did not disclose the location or source of their data collection, but identified the patients as injured in combat.(29)

In general, the risk of bias for these studies was low. The scores for these studies ranged from 0 to 4 with a mean score of 2.86 ± 1.39 (Table 2). Military studies had a higher risk of bias, with a mean score of 3.75 ± 0.5. The most common sources of potential bias were lack of reporting of primary outcomes like mortality and length of stay, and adverse events like sepsis and acute respiratory distress syndrome, with only five investigations reporting appropriate primary outcomes (22, 30, 32, 33, 35) and five reporting suitable adverse events.(16, 22, 32, 33, 35)

The mean sample size of these studies was 424 ± 280 participants, with a range of 21 to 1,250 participants; therefore, adequate sample sizes were available for the majority of studies. A mixture of blunt and penetrating trauma patients were included among the majority of studies, with a larger portion of the study population in military studies involving penetrating injuries.(17, 24, 34) Sperry and colleagues (16) and Brown and colleagues (33) included only patients with blunt trauma. Investigators for 2 studies did not report the proportions of blunt versus penetrating trauma.(26, 29) Evidence exists to suggest that blunt trauma patients experienced different mechanisms of tissue and organ injury compared to those with penetrating trauma, with the probability of transfusion likely differing between the groups.(36, 37) Thus, comparison of findings among studies

in this review involving a mixture of patients who suffered blunt and penetrating injuries is limited, as it is highly likely that the extent of their injuries and course of treatment varied.

While the majority of investigators (81%) used the same definition of MT, 10 or more units of PRBCs in a 24-hour timeframe, (15, 17-20, 23-27, 29-35) investigators for nearly one fifth of studies (18%) defined MT differently; thus, meta-analysis was not possible. Definitions ranged from a minimum of 5 (28) to 10 (22) units of PRBCs during a period defined either by the hours following injury (e.g. the first 12 hours (16)), the hours following admission (ranging from 4 (28) to 6 (21)), or the patient location (between ED admission and transfer to the intensive care unit).(22) Thus, variation existed among investigators in the MT definition.

Investigators for 7 studies accounted for survival bias,(18, 20, 22, 23, 27, 30, 32) the concept that certain patients may have been more likely to die earlier than others because they did not live long enough to receive the treatments necessary for survival. These provisions included: exclusion of patients who died within a certain timeframe, i.e. following admission to the emergency room;(20, 27, 30, 32) exclusion based on the location of a patient when death occurred;(23) death occurring prior to admission to the intensive care unit;(22) and death prior to receipt of surgical intervention for injuries.(18) In contrast, some investigators argued that the first six hours of treatment were the most crucial to survival, as this was when coagulopathy correction occurred; thus, patients who died in this timeframe should have been included to inform the process of resuscitation and determine best practice.(21, 24)

The use of biological variables among investigators to either characterize their sample, or control for confounding variables in their analyses ranged from none (19) to 12 measures.(28) The most commonly used physiological measure was blood pressure, which was measured in 17 investigations.(15, 17, 18, 20, 22-24, 26-28, 30-35) Other biological variables less commonly used included: hemoglobin,(17, 21-24, 27-29, 32, 34, 35) blood pH,(16, 20, 21, 24, 28, 30) temperature,(16, 17, 20, 21, 24, 28-30, 33, 34) heart rate,(17, 20, 24, 28-32, 34) respiratory rate,(28, 34) coagulation factors,(17, 20-25, 27-35) base excess/deficit,(16, 17, 20-25, 29-35) lactate,(21, 28, 31, 33, 35) platelet count,(17, 20, 22, 24, 25, 27, 28, 30-32, 34, 35) white blood cell count,(22) fibrinogen,(27, 28) and bicarbonate level.(28) Such variation limited the ability to compare patients and the corresponding findings from the analyses.

In summary, there was a low risk of bias among the studies included in the review. However, comparison of findings across studies remained difficult due to several factors. Inclusion of both blunt and penetrating trauma patients in the majority of studies posed a challenge, as mechanisms of injury and the subsequent course of treatment and recovery may differ between the injury groups. Though the majority of investigators used the same MT definition, roughly 20% did not. Existing controversy surrounding survival bias analysis techniques further limits comparison of findings given the removal of patients from certain analyses based on early death. Finally, biological measures were not consistently reported among investigators; thus, there was an incomplete clinical picture of patients related to transfusion requirements and clinical outcomes.

Results

Ratio of Blood Components Administered

Prior research evidence suggested that whole blood was superior to blood components for resuscitation of trauma patients, and that transfusion of a 1:1:1 ratio of PRBCs to FFP to PLTs was the closest approximation to whole blood, and was superior to the use of single components, or multiple components in a different ratio for resuscitation.(38-40) The ratio of blood components administered was used in these studies to group patients, and evaluate outcomes associated with the ratio of units of FFP to PRBC units, or units of PLTs to PRBCs. None of the MT protocols reported in these studies included prescription for transfused component ratio. The methods by which investigators chose ratio groupings for comparison purposes was largely arbitrary, with only five investigators citing former studies,(19, 24, 33-35) and investigators for two studies referred to their ratio groupings as clinically relevant without definition.(15, 27)

Investigators categorized ratios as high, medium, or low, used a numerical range, or included both strategies. A low ratio of FFP to PRBC ranged from < 1:1.5 (25, 31-33, 35) to < 1:18 (15) indicating that for every unit of FFP, 1.5 to 18 units of PRBCs were administered. The medium range included values between > 1:8 to $\leq$ 1:3,(26) $\geq$ 1:18 to < 1:12,(15) and $\leq$ 1:1.5 to $\geq$ 1:2;(35) this, again, translated into administration of 1 unit of FFP for every 1.5 to 18 units of PRBCs. There was obvious overlap with the low ratio group in some categorization schemas. The high ratio ranged from $\geq$ 1:2,(25, 31, 32) to $\geq$ 1:12 to < 1:6,(15) or < 1:2,(35) which would indicate that for every unit of FFP, < 2 to 12 units of PRBCs were administered. The high ratio was considered the closest to the 1:1 ratio intended to mimic whole blood; however, the variability in this definition was quite

large. Investigators for two military studies defined a low ratio as < 1:4,(29) or 1:8;(17) the high ratio was defined as > 1:2, and 1:1.4, respectively, indicating that for every unit of FFP, the patient received anywhere from 1.4 to 8 units of PRBCs. Given the wide range in definition of ratio groups and administration of components, comparison of findings among patients is limited.

The categorization of PLT:PRBC ratios was similar to that of FFP:PRBCs. Low ratios, the closest to the 1:1:1 ratio, ranged from < 1:18 (15) to < 1:2,(20) with one group of investigators using > 1:20 (30) as a low ratio category, which indicated that for each unit of PLTs, the patient received 2 to 18 units of PRBCs. Thus, these categorization strategies were also inconsistent. Few investigators used a medium range for PLT:PRBC ratios.(15, 24, 27, 30) Of those investigators who reported a medium ratio range, ratio values included $\geq$ 1:18 to < 1:12,(15) 1:16 to < 1:8,(24) $\geq$ 1:4 to 1:1,(27) and 1:2, meaning that patients received 1 to 18 units of PRBCs for each unit of PLTs received.(30) High ratios were equally as varied, and were defined as anywhere from $\geq$ 1:1(27) to $\geq$ 1:12 to < 1:6, indicating that patients received 1 to 12 units of PRBCs for every unit of PLTs.(15) Inaba and colleagues (15) were the only investigators to include a highest ratio group, which consisted of a ratio > 1:6, which indicated that patients received more than 1 unit of PLTs for every 6 units of PRBCs transfused. As a result of the wide variety of ratio groupings and the lack of consistency in the methods by which groups were chosen, comparisons of outcomes based on component ratio was not possible.

MT and Outcomes

Association of component ratio and mortality

Mortality was evaluated at 6,(22, 27, 31-35) 12,(15, 33) and 24 hours,(15, 16, 22, 24, 25, 27, 28, 30, 32-35) 30 days,(19, 22, 24, 27, 28, 30, 32, 34) and/or at hospital discharge.(15-18, 20, 21, 23, 25-29, 31, 32, 35) For the purpose of our review of findings, the mortality results from the latest point in hospitalization are presented and summarized from investigators who analyzed this outcome at multiple times. Ten of the 17 investigators (59%) who focused on FFP:PRBC ratios ultimately concluded there was significant survival benefit when the FFP:PRBC ratio neared 1:1; the decrease in mortality ranged from 4% to 64%.(17-20, 22, 23, 26, 27, 32, 33) (See Figure 2.) In contrast, investigators for seven studies (41%) found no difference in mortality based on FFP:PRBC ratio groups.(16, 21, 25, 28, 29, 31, 35) Holcomb and colleagues (20) found that a FFP:PRBC ratio of $\geq$ 1:2 was associated with a 20% higher 30-day survival among a mixture of military and civilian patients; while Borgman and colleagues (17) identified an average mortality reduction of 46% when military patients received a ratio closer to 1:1 compared with those who received a ratio further from 1:1. In contrast, Van and colleagues found no difference in mortality based on component ratio in military trauma patients.(29)

Investigators who studied the association of PLT:PRBC reported similar findings; investigators for seven of the eight studies (88%) concluded that there was superior survival for both military and civilian patients who received PLT:PRBC ratio closest to 1:1.(15, 19, 20, 24, 27, 30, 33) (See Figure 3.) Differences in mortality rates between ratio groups were less dramatic than those reported for FFP:PRBC ratios, and ranged

from 8%(33) to 49%.(15) Only one group of investigators did not find a significant difference in mortality between high and low ratio groups.(34) Interestingly, this study included only combat casualties. These investigators reported an inability to perform time-dependent analyses, and a lack of data to permit control of crystalloid infusion volume; thus, the difference in findings may be a function of lack of control for confounding variables.(34) In summary, administration of blood components close to 1:1:1 for PRBCs:FFP:PLTs was significantly associated with reduced mortality at 6,(22, 27, 32, 33) 12,(15, 33) and 24 hours,(15, 22, 24, 27, 30, 32, 33) 30 days,(19, 22, 24, 27, 30, 32) and at hospital discharge in the majority of these studies.(15, 17, 18, 20, 23, 26, 27, 32)

Association of component ratio and clinical outcomes

Clinical outcomes in addition to mortality were evaluated by investigators for 15 of 21 studies (71%).(16, 17, 20, 22-25, 27-30, 32-35) Investigators in 9 studies focused on outcomes for those receiving FFP:PRBC high and low ratios;(16, 17, 22, 23, 25, 28, 29, 32, 35) three other studies were focused on PLT:PRBC ratios,(24, 30, 34) and another three studies were focused on patients who received both FFP:PRBC and PLT:PRBC ratios.(20, 27, 33) The most frequently reported outcomes among these 15 investigations included multiple organ failure,(16, 17, 20, 22, 24, 25, 30, 32-34) intensive care unit (ICU) length of stay, alternatively reported as ICU-free days,(16, 20, 22, 23, 28, 30, 32, 33, 35) hospital length of stay, alternatively reported as hospital-free days,(16, 20, 22, 28, 30, 32, 33, 35) and ventilator days or hours, alternatively reported as ventilator-free days or hours.(16, 20, 22, 27, 28, 30, 32)

Investigators for 3 studies found significant differences in rates of multiple organ failure between a high ratio (closer to 1:1) and a low ratio (two FFP:PRBC studies, one PLT:PRBC study).(22,30,34) Those in the low ratio groups experienced an average multiple organ failure rate of 27%, compared to those in the high groups who experienced an average rate of 47%.(22, 30, 34) Investigators for 4 studies found significant differences between FFP:PRBC ratio groups, with those who received high ratios experiencing hospital lengths of stay averaging 15.5 days longer than those in the low ratio groups.(16,22,32,33) Holcomb and colleagues reported a greater average of hospital-free days for those who received a combination of high FFP:PRBC and high PLT:PRBC ratios (6 ± 8 days) compared with those who received high FFP:PRBC but low PLT:PRBC ratios (3 ± 6 days), and for those who received a combination of low FFP:PRBC with high PLT:PRBC (5 ± 8 days) compared with those who received low FFP:PRBC but low PLT:PRBC ratios (3 ± 7 days, $p < 0.001$ across all groups).(20)

The average ICU length of stay reported in days was 15.5 ± 4.4 for high ratio groups and 14.1 ± 6.3 days for low ratio groups; average ICU-free days numbered 7.5 ± 3.5 days for high ratio groups and 5.5 ± 3.5 days for low ratio groups. However, findings related to ICU length of stay related to ratio groups in individual studies were mixed. Investigators for four studies reported a greater length of stay averaging 4.6 days longer for those who received a high FFP:PRBC ratio (closer to 1:1) compared with those who received a low ratio,(16, 22, 28, 32) and one investigator for another study reported greater length of stay for those in the high PLT:PRBC ratio group versus the low (high 18 days, low 15 days, $p < 0.01$).(33) However, more ICU-free days (shorter length of stay) were reported for patients who received high ratios for both FFP:PRBC and PLT:PRBC

(average 5 ICU-free days) compared with low (average 3 ICU-free days),(20) as well as for those who received high PLT:PRBC only (high, average 10 ICU-free days vs low, average 8 ICU-free days).(30)

Findings related to ventilation time were similarly varied. Investigators in 7 studies reported significant differences in ventilation duration between high and low ratio groups. The average number of ventilator days was 12 ± 3.6 for those in high ratio groups and 7.8 ± 5.6 days for those in low ratio groups;(16,22,28) patients in the high ratio groups experienced an average of 9.5 ± 2.9 ventilator-free days compared to an average of 6 ± 2.9 ventilator-free days for those in low ratio groups.(20,27,30,32) Four of the 15 investigations (27%) identified no difference between the ratio groups for any clinical outcome evaluated.(17, 24, 25, 35)

Therefore, while administration of components in a 1:1:1 fashion may be more beneficial to survival, those who do survive could experience greater complication rates, hospital/ICU length of stay, and greater ventilator days. Greater complication rates and lengths of stay may be due to the severity of illness and/or the presence of comorbidities further complicating severity of illness. Alternatively, shorter lengths of stay and ventilation times for those in the low ratio groups may be the result of early deaths due to clinical complications or severe illness. Further research is required to fully understand this relationship.

Discussion

MT protocols are still fairly new among trauma centers, but may be beneficial in guiding treatment of critically injured patients. Evidence exists to support a decrease in mortality,(41, 42) length of stay,(42) and ventilator days (42) with the use of MT

protocols in resuscitation of trauma patients. Though guidelines from the American College of Surgeons are available to aid practitioners in their methods of transfusion and development of MT protocols,(43) no standardized MT protocol is used across trauma centers. As a result, protocols lack consistency in the physiological requirements for activation and cessation of component delivery, the clinicians who may activate them or stop delivery of components, and the amounts of components delivered to the bedside upon activation. Furthermore, protocols do not prescribe ratios of components to be transfused, only the amounts released from the blood bank upon activation. Some investigators argue, however, that the treatment prescribed by MT protocols may not be applicable for all massively hemorrhaging patients;(44) thereby removing the need for consistency among protocols and allowing clinician discretion in administration of components as needed.

The most effective ratios of components transfused during MT for survival and outcomes benefits remains controversial as well. Our review suggested transfusion of components in a 1:1:1 fashion may produce superior survival in trauma patients, but could also result in prolonged lengths of stay and increased complications. Neal and colleagues (45) concluded that the evidence to support a 1:1:1 ratio of components was "impressive", and recommended the use of a 1:1:1 ratio in resuscitation practices.

Investigators have found similar results in patients with both blunt and penetrating trauma, with those receiving closer to a 1:1:1 ratio experiencing better outcomes.(37) The same group of investigators suggested a more beneficial effect of the 1:1:1 ratio in male trauma patients compared with female, possibly as a result of inherent hormonal differences.(47) Mixed findings existed in the current analysis with regards to the three

military studies, potentially the result of different injury patterns and treatment methods, as compared to those experienced by civilian patients. Thus, further investigation is warranted to determine best practice in general in patients with trauma, and more specifically between the genders and in military versus civilian populations.

Studies included in this review were primarily retrospective or observational, with minimal bias issues. Two of the greatest sources of bias were in the reporting of primary outcomes and adverse events. This may be due to the wide range of outcomes and adverse events patients transfused after trauma experienced during hospitalization. The third source of bias was a lack of control of confounding variables in analyses, found in 12 (57%) of the studies. This could be the result of limited data availability or missing data given the nature of retrospective studies, and the chaotic atmosphere surrounding trauma resuscitation. Alternatively, some investigators may have chosen to exclude confounding factors for unknown reasons.

In either case, a lack of confounding factors in over half of the studies greatly impaired comparison of findings. For example, Riskin and colleagues (47) included 10 variables in their prediction model, including transfusion-related variables, age, and injury characteristics. Similarly, Perkins and colleagues included 12 variables much like those used by Riskin et al., but these investigators added laboratory coagulation values and body temperature.(24) In contrast, Gunter and colleagues used only three regression variables: Trauma Related Injury Severity Score, age, and the intra-operative ratio of transfused components.(19) More consistency among investigators in control of confounding variables in analyses is critical for valid findings and interpretations.

Another factor to be considered is the effect of survival bias. Survival bias presents major issues in data analysis and associated findings, especially in trauma resuscitation literature.(48) Though exclusion of patients prior to a certain time point (e.g. the first 30 minutes following admission) provides a more homogeneous sample, this also eliminates a specific type of patient/experience that may be vital to understanding the population and phenomenon of interest. Investigators suggest the use of time-dependent analyses such as Cox proportional hazards models to help reduce survival bias, using timeframes in which no patients died (e.g. total transfused volume in the first 24 hours following injury).(49) Use of such techniques may lead to alternate findings compared to more traditional techniques like logistic or linear regression. In fact, several investigators cited in this study found conflicting results in their own analyses, noting a survival benefit initially, but finding no survival benefit once adjusting for survival bias.(25, 31) Other investigators note the importance of including confounding factors related to transfusion but not involving the amount or ratio of components transfused that may be associated with patient survival, such as changes in hospital protocol calling for reduced crystalloid infusion or mandating earlier transfusion of certain components.(48)

Though the findings in this review support 1:1:1 transfusion, it should be emphasized that all studies reviewed were retrospective or observational, and not all investigators found an increased survival benefit with this combination of components. In fact, it is due to the nature of studies such as these and the issues with survival bias that the Canadian National Advisory Committee on Blood and Blood Products determined in 2011 that the evidence was not sufficient to warrant changes in their massive transfusion treatment recommendations to include a 1:1:1 transfusion ratio.(50) Randomized clinical

trials are thus required to determine best practice for this population; however, these are often time-sensitive and consume a tremendous amount of resources. Though few studies of this nature have been performed in patients with trauma who require massive transfusion due to economic, ethical, and logistic challenges, investigators believe it is possible despite these obstacles.(51, 52)

Conclusion

The majority of evidence in our review supported transfusion of packed red blood cells, fresh frozen plasma, and platelets in a 1:1:1 fashion in massively hemorrhaging patients after trauma for superior survival. Clinical outcome benefits remain equivocal and require further investigation. Randomized controlled trials are necessary to adequately address transfusion practice in this population.

Acknowledgments

None

Table 3.1 Bias Assessment Instrument*

Criteria	Scoring Options
Inclusion/exclusion criteria across groups	Not applicable (0) No, does not vary across groups (0) Partially varies across groups (1) Cannot determine (2) Yes, varies across groups (3)
Selection of appropriate comparison group	Not applicable (0) Not inappropriate (0) Cannot determine (1) Yes, inappropriate (2)
Valid/reliable measures used across groups	Yes, valid/reliable measures used (0) Cannot determine or not reported (1) No, valid/reliable measures not used (2)
Length of follow-up across groups	Not applicable (0) No, not different; remedied through analysis (0) Yes, different or cannot determine (1)
Important outcomes missing from results	No, none missing (0) Cannot determine (1) Yes, some missing (2)
Important harms or adverse events missing from results	No, none missing (0) Not applicable to this study (0) Yes, some missing (1)
Results believable	Yes, believable (0) No, not believable (1)
Attempts to balance groups	Not applicable (0) Yes, accounts for imbalance statistically (0) No or cannot determine (1)
Confounding variables missing from design	No, taken into account (0) Partially – some included (1) Cannot determine (2) Yes, not accounted for or not identified (3)

* Instrument based on Viswanathan M, Berkman ND. Development of the RTI item bank on risk of bias and precision of observational studies. J Clin Epidemiol. 2012 Feb; 65(2):163-78.

Table 3.2 Description of Included Studies (n = 21)

Citation	Setting	MT Protocol/Definition	Sample	Methods	Findings	Quality Score
Borgman et al. (2007)	Unidentified Combat Support Hospital, Iraq	- MT definition: 10 ≥ units PRBC or fresh whole blood within 24 hours from admission - FFP:PRBC ratio groups chosen based on median ratios determined by bootstrapping technique: low (1:8), medium (1:2.5), high (1:1.4) - No MT protocol identified	- Trauma patients admitted to combat support hospital who received a MT - N = 246; 94% penetrating injuries; 3 female patients; median age 24 years; 28% mortality	Retrospective review of admissions in the Joint Theater Trauma Registry from between November 2003 – September 2005	- Mortality of low, medium, and high groups were 65%, 34%, and 19%, respectively ($p < 0.001$) - Low plasma ratio group did not receive platelets; platelets only used in 27% of patients - Median time of death measured in hours from admission to the hospital was 2 hours in the low group (IQR 1-4), 4 hours in the medium group (IQR 2-16), and 38 hours in the high group (IQR 4-155) ($p < 0.001$) - FFP:PRBC ratio independently associated with overall survival (OR 8.6, 95% CI 2.1 – 35.2, p = 0.003), as was base deficit (OR 0.89, 95% CI 0.84 – 0.95, $p < 0.001$)	3
Duchesne et al. (2008)	Charity Hospital, New Orleans, LA	- MT definition: > 10 units PRBC in first 24 hours of admission - FFP:PRBC ratio groups: patients grouped into those who received ≤ 10 units PRBC and those who received > 10 units PRBC during the first 24 hours following injury; further divided into those receiving FFP:PRBC ratio of 1:1 and 1:4 - Protocol activated by trauma surgeon; calls for 6 units PRBC, 6 FFP, 6 platelets, and 10 of cryoprecipitate	- Adult trauma patients who required surgical intervention; Patients with ≤ 10 units PRBC (N = 250): 31 ± 13, 85% male, 58% penetrating injuries, 17% died; Patients with > 10 units PRBC (n = 135): 33 ± 11, 86% male, 72% penetrating injuries, 56% died	Retrospective review of admissions to trauma intensive care unit between January 2002 and December 2006	- In patients who received > 10 units PRBC, significant difference in mortality in those patients with a 1:4 ratio versus those with a 1:1 ratio (87.5% vs 26%, $p = 0.0001$)	4

Table 3.2, Cont.

Gunter et al. (2008)	Vanderbilt University Medical Center, Nashville, TN	- MT: ≥ 10 PRBC in first 24 hours - Groups separated for analysis based on FFP:PRBC ratio: 0:1 – 1:2.9; 1:3 – 1:1.49; 1:1.5 – 0.9:1; ≥ 1:1 - 24-hr blood product use calculated; patients separated into groups based on FFP:PRBC and PLT:PRBC ratios intraoperatively and for first 24 hours after admission; outcomes evaluated for those with FFP:PRBC ratio of ≥ 2:3 and PLT:PRBC ratio of ≤ 1:5; cut-points chosen based on previous studies - Attending trauma surgeon activates Trauma Exsanguination Protocol (TEP) - Protocol includes blood product ratio of: 10 units PRBC, 4 units AB negative FFP, 2 units PLT	- Pre-TEP: n = 140, median age: 36 years (24-50), 76% male, 61% penetrating injuries; Post-TEP: n = 119, median age: 30 (24-43), 75% male, 48% penetrating injuries	Retrospective review of post-TEP protocol initiation admissions between Feb. 1, 2009 and July 31, 2007; Comparison cohort of pre-TEP admissions selected from trauma admissions between August 1, 2004 and January 31, 2006	- Both FFP:PRBC and PLT:PRBC ratios higher (closer to 1:1) for survivors - Patients with intraoperative and 24-hr FFP:PRBC ratio ≥ 2:3 more likely to survive -Patients who did not achieve a PLT:PRBC ratio of ≥ 1:5 less likely to survive - Lowest 30-d mortality was intraoperative FFP:PRBC ratio between 1:1.5 – 1:1.01; significantly less than mortality rates for all other ratio ranges ($p <$ 0.001)	4
Holcomb et al. (2008)	16 level-I trauma centers in the United States	- MT definition: ≥ 10 units PRBC in 24 hours - Patients categorized by FFP:PRBC ratio and PLT:PRBC ratio - FFP:RBC ratio groups included low (<1:2) and high (≥ 1:2) - PLT:PRBC ratio groups included low (<1:2) and high (≥ 1:2) - Patients further grouped based on both FFP:PRBC and PLT:PRBC (1: high-high; 2: high-low; 3: low-high; and 4: low-low) - No protocols specified for any of the 16 centers	- Patients who arrived from the scene of the trauma, and who received at least 1 unit PRBC in the emergency department, irrespective of mechanism of injury (N = 467); - Excluded those who died within 30 minutes of arrival (n = 1) leaving a total of 466 MT patients for analysis - Mean age 39 ± 18; 76% male; 65% blunt injury	- Retrospective multi-center review of transfused trauma patients admitted between July 2005 and June 2006	- A FFP:PRBC ratio of ≥ 1:2 was associated with improved 30-day survival (40.4% vs 59.6%, p < 0.01) - A PLT:PRBC ratio of ≥ 1:2 was associated with improved 30-day survival as well (40.1% vs 59.9%, p < 0.01) - Group 1 (high-high ratios) had increased survival compared with the other 3 groups at 6 and 24 hours (p < 0.001) - Those who received high PLT:PRBC and FFP:PRBC ratios had increased ventilator-free, ICU-free, and hospital-free days (p < 0.001) compared to those who received low PLT:PRBC and FFP:PRBC ratios	4

Table 3.2, Cont.

Kashuk et al. (2008)	Denver Health Medical Center (DHMC), Denver, CO	- MT definition: > 10 units PRBC in first 6 hours after admission - FFP:PRBC ratio divided into 1:1, 1:2, 1:3, 1:4, and ≥ 1:5 - No formal protocol in place at time of review	- All patients undergoing massive transfusion; N = 133; mean age 34.9 ± 17.1; overall mortality 56%; death from penetrating trauma 41%; death from blunt trauma 59%	- Retrospective review of trauma center's registry and transfusion registry maintained by blood bank including patients admitted to DHMC from 2001 to 2006	- Multiple logistic regression significant independent variables (p < 0.05, OR 95%): PRBC units transfused at 6 hours (OR = 1.248, 1.038-1.051), ED temperature < 34° (OR = 15.491, 1.376 – 174.396), age > 55 years (OR = 40.531, 5.315 – 309.077) **instead of a linear correlation, followed a U-shaped relationship with death as an end point - Median FFP:PRBC ratio for all survivors was 1:2, and for non-survivors, 1:4 - > 80% of transfusion requirements were completed within the first 6 hours after ED admission	3
Maegele et al. (2008)	70 hospitals in Germany contributing to the Trauma Registry of the Deutsche Gesellschaft für Unfallchirurgie (TR-DGU)	- MT definition: minimum of 10 units PRBC between ER arrival and ICU admission - Ratio definitions: Group 1 (PRBC:FFP – **ONLY STUDY TO REVERSE RATIO**) > 1:1; Group 2 (PRBC:FFP 0.9-1.1 or 1:1); Group 3 (PRBC:FFP < 0.9 - No protocol identified	- Primary admissions to the trauma center; patients with ISS ≥ 16, who met definition of MT (N = 713) - 69.7% male; mean age 40.1 18; mortality Group 1 45.9%, Group 2 36%, Group 3 30.4%; blunt injury Group 1 92.3%, Group 2 87.7%, Group 3 97.4%	- Retrospective review of data collected from TR-DGU from 2002 to 2006	- All mortality rates (6 hr, 24 hr, 30-day) were highest among Group 1 (PRBC:FFP >1.1) - Acute (< 24 hr) and 30-day mortality were lowest in patients who had a PRBC:FFP ratio < 0.9	1

Table 3.2, Cont.

Sperry et al. (2008)	Seven institutions across the US	- MT definition: ≥ 8 units PRBC in first 12 hours after injury - Ratio definitions for FFP:PRBC were: high (≥ 1:1.5), low (< 1:1.5) - Low ratio group further broken down: 1:2 (1:1.51 – 1:2.5), 1:3 to 1:4 (1:2.51 – 1:4.5), and ≤ 1:5 (≤ 1:4.51) - No protocol identified	- Patients required ≥ 8 units PRBC within the first 12 hours after injury, blunt mechanism of injury, presence of pre-hospital or emergency department systolic hypotension or an elevated base deficit, any body region exclusive of the brain with an abbreviated injury score ≥ 2, between ages of 16 and 90 - N = 415; median age 41 (IQR 25-54); 76.5% male in the high group, 68.4% male in the low group; overall mortality 33.5%; median amount units blood transfused in first 12 hours for entire cohort 14 units (IQR 14-22)	- Prospective cohort study: Inflammation and the Host Response to Injury Large Scale Collaborative Program supported by the National Institute of General Medical Sciences; patients admitted between November 2003 – March 2007	- No statistically significant differences found in mortality between those receiving the high ratio and those receiving the low - Those who received a high ratio had a reduced blood transfusion requirement at 12 and 24 hours post-injury (p = 0.001) -Survival curves overall were not statistically significant; however, the mortality rate at day 1 post-injury was significantly lower in the high ratio group (3.9% vs 12.8%, p = 0.012) - Those who received a high FFP:PRBC ratio had a 52% decrease in the likelihood of mortality (HR 0.48, p = 0.002, 95% CI 0.3 – 0.8); variation of the model to include only patients who received between 4-8 units of blood did not reach significance, indicating that the effect of the ratio may only apply to those being massively transfused	2

Table 3.2, Cont.

Duchesne et al. (2009)	Unidentified Level-I urban trauma center in the United States	- MT definition: ≥ 10 units PRBC within 24 hours - Patients grouped according to ratio of FFP:PRBC (1:1, 1:2, 1:3, 1:4) – no designation of "high" or "low" groups - MT protocol: 6 units FFP and 6 units PRBC available in the trauma bay; protocol activated by trauma surgeon	- Patients with initial trauma induced coagulopathy diagnosed on arrival to the emergency department, who required FFP and > 10 units PRBC in the OR during initial surgical intervention - N = 135; mean age 35; 88.7% male; mean ISS 21.7; 68.7% penetrating trauma; 1:1 group – 46 (34.1%), 1:2 group – 26 (19.2%), 1:3 group – 20 (14.8%), 1:4 group – 43 (31.8%)	- Retrospective review of trauma patients admitted between January 2001 to December 2007	- 31% of population diagnosed with trauma-induced coagulopathy on arrival - No significant differences in demographic or clinical variables among groups - Patients in 1:4 group experienced 13 day increase in Trauma ICU length of stay compared with 1:1 group ($p < 0.01$) - Overall mortality per transfusion group: 1:1 – 28.3%; 1:2 – 38.5%; 1:3 – 40.0%; 1:4 – 51.2% - Overall mortality differences statistically significant between 1:1 group (28.2%) and 1:4 group (51.1%) ($p = 0.03$) - Mortality in operating room for 1:1 group was 8.7% vs 34.9% in 1:4 group ($p < 0.01$); mortality was similar between these groups after arrival to Trauma ICU - Patients in the 1:3 and 1:4 ratio groups were 3.76 ($p = 0.03$, 95% CI 1.18-11.9) and 4.17 ($p < 0.01$, 95% CI 1.48 – 11.7) more likely to die in the operating room; risk of dying was not significantly different between groups after Trauma ICU admission	3

Table 3.2, Cont.

Perkins et al. (2009)	US Army Combat Support Hospital at Ibn Sina Hospital in Baghdad, Iraq	- MT definition: ≥ 10 units of blood within 24 hours - Patients grouped based on ratio of PLT:PRBC; low ratio (< 1:16), medium (1:16 to < 1:8), and high (≥ 1:8) - MT protocol in place to guide resuscitation; not specified further	- Patients who received ≥ 10 of blood within 24 hours; excluded those who received fresh whole blood - N = 464; Median age 27-28; > 95% male, median ISS 20 – 21, > 90% penetrating trauma; 214 patients were in the low ratio group, 154 in the medium, and 96 in the high	- Retrospective review of trauma patients admitted between January 2004 – December 2006	- Survival at 24 hours was 64%, 87% and 95% in the low, medium, and high ratio groups respectively (χ^2, $p < 0.001$ for low versus medium and high PLT ratio group comparisons, χ^2, $p = 0.04$ for medium versus high PLT ratio group comparisons); most deaths in the first 24 hours occurred within the first 6 hours after admission - 30-day survival differed between low (42.7%), medium (60%), and high (75%) groups as well ($p < 0.001$, log rank comparison) - Variables independently associated with decreased mortality at 24 hours included: FFP ratio (OR, 0.94, $p < 0.001$, 95% CI 0.91 – 0.96), PLT ratio (OR, 0.82, $p = 0.002$, 95% CI 0.72 – 0.93)	4
Snyder et al. (2009)	University of Alabama-Birmingham Hospital, Birmingham, AL	-MT: ≥ 10 units of PRBC during the first 24 hours of admission - No ratio groups pre-defined - No protocol in place	- All trauma patients massively transfused (N = 134); Survivors (n = 67) - mean age 36.8, 64.2% male, 40.3% blunt injury; Non-survivors (n = 67) – mean age 41.6, 77.6% male, 38.8% blunt injury	- Retrospective chart review of admissions between January 2005 and January 2007;to calculate ratio of blood products used in resuscitation; formula used: FFP:PRBC ratio = F/(B+C) where C represents total auto-transfused cell saver units	- 68% of 24-hour totals were given in the first 6 hours of admission; 92% in the first 12 hours - First PRBC unit given at a median of 18 mins; first FFP unit given at a median of 93 mins - Patients in high-ratio group had a significantly lower risk of death compared with low-ratio group (RR, 0.37; 95% CI 0.22-0.64) - Majority of deaths for low-ratio group occurred within the first 6 hours of admission; majority of deaths for high-ratio group occurred after initial 24 hours	3

Table 3.2, Cont.

Teixeira et al. (2009)	Los Angeles County + University of Southern California Medical Center, Los Angeles, CA	- MT: ≥ 10 units PRBC during the initial 24 hours after admission - Four groups of FFP:PRBC ratios: Low (≤ 1:8), Medium (> 1:8 and ≤ 1:3), High (> 1:3 and ≤ 1:2), Highest (> 1:2) - No MT protocol in place during study period	- Trauma patients receiving MT without severe head injury; N = 383; mean age 32 ± 15; 87% male;	- Retrospective study using Trauma Registry and the Blood Bank Database, between 2000-2005	- Mean units FFP per patient increased 83% from 2000 to 2005 (not statistically significant, p = 0.07) - FFP:PRBC second most important predictor of outcome after GCS on admission (Adj OR = 0.02, 95% CI 0.01 – 0.07, R^2 = 0.47, ROC = 0.86) - Increased likelihood of death if the FFP:PRBC ratio ≤ 1:3 - Mean ratio for survivors was 1:2.1 compared with 1:3.7 for non-survivors (p < 0.001)	4
Zink et al. (2009)	16 level-I trauma centers across the US	- MT: ≥ 10 units PRBC in the first 24 hours after injury - Ratio definitions for both FFP:PRBC and PLT:PRBC were: Low (< 1:4), Medium (≥ 1:4 to 1:1), High (≥ 1:1) - No protocol specified	- All trauma patients age 16 and older who received any PRBCs within 24 hours of admission; excluded those who died within 30 minutes of arrival - N = 452; mean age for FFP:PRBC patients ranged from 28.3 - 36; for PLT:PRBC patients ranged from 34-36; > 70% male for both FFP:PRBC and PLT:PRBC groups; overall mortality 41%	- Retrospective review of trauma patient records from those injured between July 2005 and June 2006	- Patients who received a FFP:PRBC ratio of < 1:1 within the first 6 hours after admission required a median of 18 units of PRBC throughout the first 24 hours; those who received a ratio of ≥ 1:1 only required a median of 13 units PRBC (p < 0.001) - No significant differences seen in respiratory outcomes based on ratio of FFP:PRBC or PLT:PRBC	4
Inaba et al. (2010)	Los Angeles County + University of Southern California Medical Center, Los Angeles, CA	- MT: ≥ 10 units PRBC during initial 24 hours after admission - aPLT:PRBC Ratio definitions defined: Low (< 1:18), Medium (≥ 1:18 to < 1:12), High (≥ 1:12 to < 1:6), and Highest (≥ 1:6) - No protocol identified	- All trauma patients receiving a PRBC transfusion; N = 657; 12.6% ≥ 55 years; 83.6% male; 54.8% penetrating trauma; Low ratio N = 171; Medium ratio N = 77; High ratio N = 249; Highest ratio N = 160	- Prospective study; 9-year observation period ending December 2008	- As the aPLT ratio increased, a step-wise decrease in mortality was seen (72.1% to 33.1%, adj. p < 0.001) - aPLT:PRBC ratio independently associated with improved survival at 24 hours (OR 0.92, CI 0.89-0.95), (R^2 = 0.54) - Highest ratio group 14.2% 12-hr mortality vs Low ratio group 48.2% 12-hr mortality (adj. p < 0.001)	3

Table 3.2, Cont.

Mitra et al. (2010)	Alfred Emergency & Trauma Centre, Victoria, Australia	- MT: ≥ 5 units PRBC transfused within the first 4 hours - FFP:PRBC ratio groups: >1:1.5, >1:2.5 to 1:1.5, >1:3.5 to 1:2.5, ≤ 1:3.5 - MT protocol: 4 units PRBCs and 4 units (600 mL) of FFP (1:1 ratio) – was not audited or formally used during study period	- All trauma patients who presented to the emergency department and received MT (N = 331) - Mean age 42.1 ±19.3, 86.4% blunt trauma, 68.3% suffered head injuries, 29.9% mortality	- Retrospective review of patient records from the Alfred Hospital database from July 2004 – August 2008	- No significant differences in mortality among the ratio groups - The FFP:PRBC ratio in the first 4 hours was not significantly associated with overall mortality	4
Van et al. (2010)	No location specified; protocol approved by Institutional Review Board at Brooke Army Medical Center	- MT: > 10 units PRBC in 24 hours; one unit fresh whole blood (FWB) equivalent to one unit PRBC and one unit FFP - Groups based on FFP:PRBC ratio: low (<1:4), mid (1:4 to 1:2), high (>1:2) - No protocol identified	-All patients with isolated extremity injuries who received at least 1 unit PRBC (N = 703) - MT: mean age 25.7, heart rate 117, temperature 97.6, base deficit 8.0; Non-MT: mean age 25.8, heart rate 99, temperature 98.2, base deficit 3.7 - MT mortality 17.2% (low ratio), 8.5% (mid ratio), 6.9% (high ratio)	- Retrospective review of database of combat-injured patients from March 3003 to June 2008	- No significant differences in mortality rates among both the MT and non-MT ratio groups - In non-MT population, those who received a low ratio had a significantly lower incidence of pulmonary embolus than those in the mid and high groups (1.4 vs 6.0 and 1.4 vs 6.7, respectively, $p < 0.05$)	4
Holcomb et al. (2011)	22 Level I Trauma Centers across the United States	- MT: ≥ 10 units PRBC within 24 hours of admission; apheresis platelet unites were converted to pooled PLT (1 aPLT unit = 6 pooled PLT units) - Ratio definitions for PLT:PRBC groups: Low (>1:20), Medium (1:2), and High (1:1) - No protocol identified	- All patients admitted directly to the emergency department from the scene of injury who received at least one unit of PRBC in the emergency department (N = 643); excluded those who received MT and died within 60 minutes of arrival - Mean age 39 ± 18; 75% male; 66% blunt trauma; mean ISS 33 ± 16; 30 day survival 60%; 24 hour survival 72%; 6 hour survival 83% - Overall survival by group: 50% (Low); 55% (Medium); 69% (High)	- Retrospective multicenter institutional review of trauma patients admitted between July 2005 and June 2006	- Increased PLT ratios were associated with improved survival at 6 hours, 24 hours, and 30 days ($p < 0.001$ for all groups) -70% of those who died within 30 days of admission died within the first 24 hours following admission; 50% died within the first 6 hours of admission - Median transfusion rates were clinically similar across the three groups indicating that patients were bleeding at approximately the same rate - Patients in the low and medium ratio groups experienced a higher risk of mortality compared with those in the high ratio group (RR = 2.81, 95% CI 1.36-5.8, p = 0.005 and RR 3.13, 95% CI 1.52-6.45, p = 0.002 respectively)	1

Table 3.2, Cont.

Magnotti et al. (2011)	Presley Memorial Trauma Center (PMTC), Shelby County, TN	- MT: ≥ 10 units of PRBC during the first 24 hours of admission - FFP:PRBC Ratio definitions: Low (< 1:2), High (≥ 1:2) - Rapid Infusion System protocol: anesthesiologist will receive 10 units each of PRBC, FFP, and PLT	- Patients admitted to the trauma center after trauma activation (N = 103); mean age 38; 69% male; 63% blunt trauma; Survivors n = 57; Non-survivors n = 46; High-Ratio n = 66; Low-Ratio n = 37	Retrospective review of the resuscitation registry at PMTC between March 2008 and December 2007	- 59% low-ratio 24-hr mortality; 38% high-ratio 24-hr mortality - 6-hr mortality was 10% in high-ratio patients vs 48% in low-ratio group ($p <$ 0.002); cause of death within the first 6 hours was uncontrolled hemorrhage - After those deaths that occurred within the first 6 hours, both groups (high- and low-ratio) were clinically similar from 6 to 24 hours	3

Table 3.2, Cont.

Peiniger et al. (2011)	116 trauma centers contributing to the Trauma Registry of the Deutsche Gesellschaft für Unfallchirurgie (TR-DGU)	- MT: ≥ 10 units of PRBC during the first 24 hours of admission - FFP:PRBC ratio groups: High (>1:2), Low (≤ 1:2) - No protocol identified	- Patients admitted to the hospital directly from scene of injury who were > 16 years of age who suffered severe injury (ISS ≥ 16 and received MT prior to ICU admission (N = 1,250); excluded those who died within first hour after admission; broken down into those with traumatic brain injury (TBI) and those without; mean age 42 ± 16 years; 72% male; mean ISS 42 ± 15; 90% blunt trauma - Overall mortality for those with TBI: Low group 62%, High group 46% - Overall mortality for those without TBI: Low group 48%, High group 27%	Retrospective analysis of all trauma patients admitted between 2002 and 2008	- 80% of patients presented with signs of coagulopathy on admission to the emergency department - Mortality rate was lower across all time points in the High group compared to the Low group, regardless of presence or absence of TBI - High FFP:PRBC (>1:2) ratio found to have survival benefit in those who suffered a TBI (OR 0.48, 95% CI 0.29-0.81, p = 0.006), but not in those who did not suffer a TBI - Those in the High ratio group experienced a greater amount of ventilator-free days compared with those in the Low ratio group; this was true for both TBI and non-TBI groups (TBI group – High ratio: 6 ± 9 days vs Low ratio: 4 ± 8 days, p = 0.006; non-TBI group – High ratio: 13 ± 12 days vs Low ratio: 9 ± 11 days, p < 0.001) - Those in the High ratio group also experienced significantly longer ICU and hospital length of stay compared with those in the Low ratio group; again, this was true for both TBI and non-TBI groups (TBI group – High ratio: 18 ± 21 days vs Low ratio: 13 ± 19 days, p < 0.001; non-TBI group – High ratio: 19 ± 20 days vs Low ratio: 15 ± 19 days, p < 0.001)	1

Table 3.2, Cont.

Brown et al. (2012)	7 institutions contributing to the Inflammation and Host Response to Injury Large Scale Collaborative Program (Supported by the National Institute of General Medical Sciences, National Institutes of Health, United States)	- MT: $\geq$ 10 units of PRBC during the first 24 hours of admission - FFP:PRBC ratio groups: High ($\geq$ 1:1.5) and Low (1:1.51-2.5) - PLT:PRBC ratio groups: High ($\geq$ 1:9) and Low (< 1:9) - A 6-pack of PLT was counted as 1 unit - No specific protocol identified; "Standard operating procedures" developed and followed by all institutions	- Patients who suffered blunt trauma only, pre-hospital hypotension (SBP < 90 mmHg) or elevated base deficit (> 6 mEq/L), blood transfusion required within first 12 hours following admission, and any body region except the head with and AIS score of $\geq$ 2; between the ages of 18-90 - N = 604; Overall mortality at 6-hours 9%, 12-hours 12%, 24-hours 13%; High FFP:PRBC group: mean age 43 $\pm$ 20; 72% male; mean ISS 40 $\pm$ 16; 6-hour mortality 4%; Low FFP:PRBC group: mean age 43 $\pm$ 18; 70% male; mean ISS 35 $\pm$ 15; 6-hour mortality 10%; High PLT:PRBC group: mean age 44 $\pm$ 18; 65% male; mean ISS 36 $\pm$ 16; 6-hour mortality 2%; Low PLT:PRBC group: mean age 43 $\pm$ 19; 72% male; mean ISS 36 $\pm$ 15; 6-hour mortality 10%	Retrospective analysis of patients admitted between 2003-2010	- Higher ratio of FFP:PRBC was associated with reduction in mortality (HR 0.19, 95% CI 0.06 – 0.56, $p < 0.01$) - Higher ratio of PLT:PRBC also associated with independent reduction in mortality (HR 0.02, 95% CI 0.01 – 0.50, $p = 0.03$) - When compared with those who received a Low FFP:PRBC ratio, those who received a High ratio of FFP:PRBC experienced a reduction of both 6-hour (HR 0.19, 95% CI 0.03 – 0.86, $p = 0.03$) and 24-hour mortality (HR 0.25, 95% CI 0.06 – 0.95, $p = 0.04$)	0

Table 3.2, Cont.

Cap et al. (2012)	Ibn Sina Hospital – United States combat support hospital in Baghdad, Iraq	- MT: ≥ 10 units of PRBC within 24 hours of admission - aPLT:PRBC ratio groups: Low (≤ 1:10) and High (> 1:10); one aPLT unit was equal to 5 units (range 4-6) of whole blood PLT - No protocol identified	- Combat casualties admitted directly from scene of injury (N = 414); Overall mortality 27%; Low group: age* 26 (21-31) 28; 99% male; ISS* 20 (12-28) 23; 93% penetrating injury; 24-hour survival 78%; 30-day survival 71%; High group: age* 26 (21-31) 28; 93% male; ISS* 25 (18-32) 24; 89% penetrating trauma; 24-hour survival 90%; 30-day survival 81%	Retrospective review of admissions between January 2004 and December 2006	- Those in the High group received more overall blood products (PRBC, FFP, aPLT, cryoprecipitate) compared with the Low group ($p < 0.01$), but also received products earlier than those in the Low group - 24-hour survival significantly greater in the High group compared to the Low group (90% vs 78% ($p = 0.02$); no significant differences between the groups at 30-days (81% vs 71%, $p = 0.08$) - Most deaths occurred within the first 6 hours following admission – 31% of those in the High group vs 68% of those in the Low group - Airway deaths (15.4% vs 3.1% Low, $p < 0.05$) and multi-organ failure syndrome (46.2% vs 16.5% Low, $p < 0.05$) more commonly occurred in the High group compared with the low group - Those in the Low group experienced a higher mortality likelihood both at 24 hours (HR 4.25, 95% CI 1.25 – 14.48, $p = 0.02$) and 30 days (HR 2.32, 95% CI 1.11 – 4.84, $p = 0.025$) compared to those in the High group	4

Table 3.2, Cont.

Kudo et al. (2013)	Tohoku University Hospital Emergency Center, Sendai, Japan	- MT: ≥ 10 units of PRBC within 24 hours of hospitalization - Ratios were calculated based on amount of components received within the first 6 hours of admission - FFP:PRBC ratio groups: High group (≥ 1:1.5), Middle (1:2 – 1:1.5), Low (< 1:2) - No protocol identified	- Trauma patients who required MT, patients who received interventions for hemostasis, surgery or trans-arterial embolization (N = 21); Overall mortality 33%; age** 60 (36.5-64.5); 67% male; ISS** 25 (17.0-34.0); 19% blunt injury; High group: 44% mortality; age** 61 (27.5-65.0); 44% male; ISS** 32 (20.5-34.0); 100% blunt injury; Middle group: 17% mortality; age** 56 (36.3-86.3); 83% male; ISS** 24.5 (15.5-27.3); 83% blunt injury; Low group: 33% mortality; age** 59.0 (35.8-61.8); 83% male; ISS** 24.5 (15.8-39.8); 83% blunt injury	Retrospective review of admissions between October 2006 and September 2009	- No significant differences among the groups for mortality (Log-Rank: $p = 0.555$), ICU-free days at 30 days ($p = 0.4$), or hospital-free days at 60 days ($p = 0.42$) - Only one patient (High group) developed sepsis; no patients developed acute respiratory distress syndrome	0

* = data presented as: median (IQR) mean
** = data presented as median (IQR)
MT = massive transfusion; PRBC = packed red blood cells; FFP = fresh frozen plasma; PLT = platelets; aPLT = apheresis platelets; IQR = interquartile range; OR = odds ratio; CI = confidence interval; ED = emergency department; HR = hazard ratio; ICU = intensive care unit; ISS = Injury Severity Score; RR = risk ratio

Figure 3.1 Article Selection

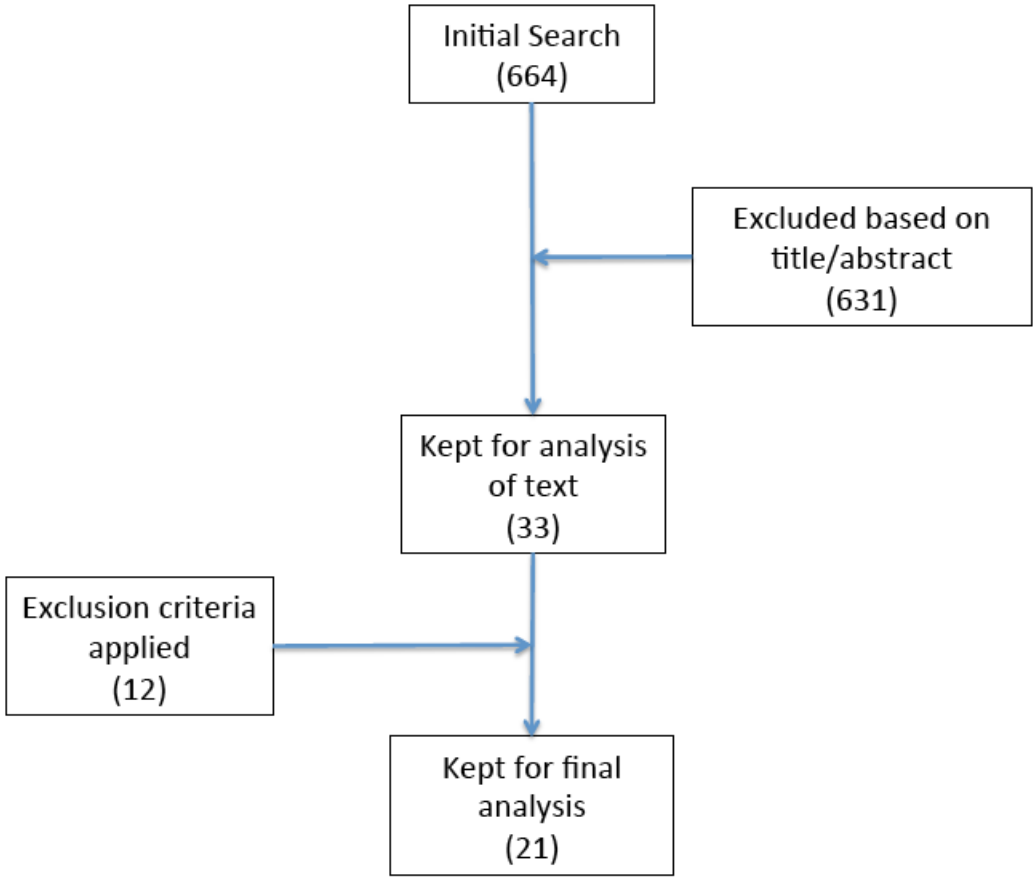


Figure 3.2 FFP:PRBC Mortality by Ratio Groups

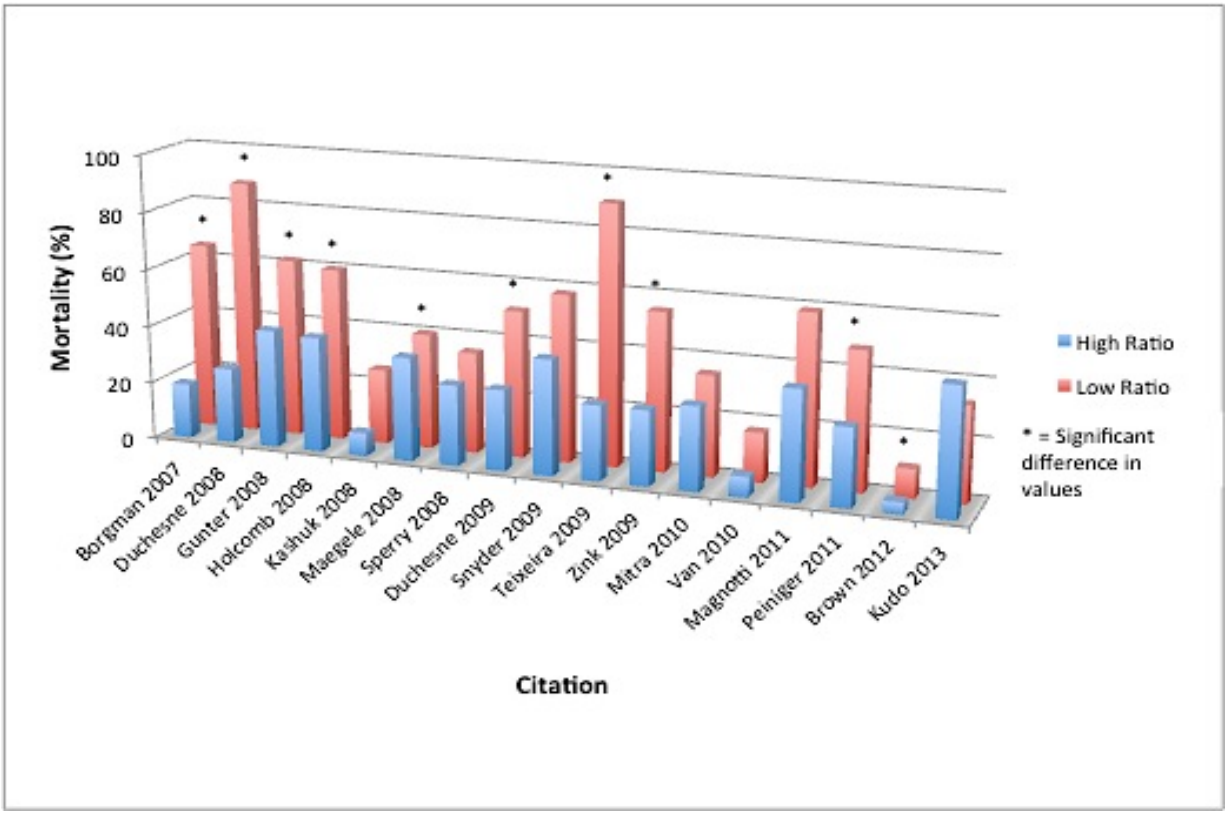


Figure 3.3 PLT:PRBC Mortality by Ratio Groups

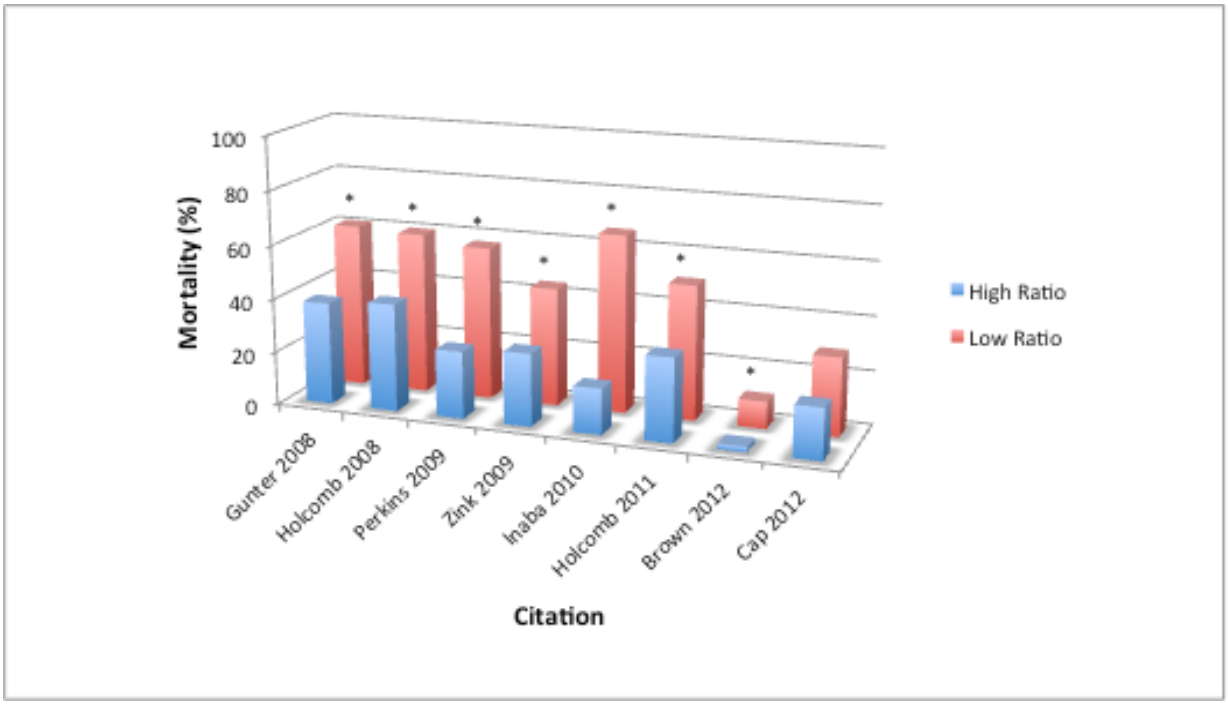


Figure Legend

Figure 3.1 Article Selection

Figure 3.2 FFP:PRBC Mortality by Ratio Group

Figure 3.3 PLT:PRBC Mortality by Ratio Group

CHAPTER FOUR

Increased Mortality in Adult Trauma Patients Transfused with Blood Components Compared with Whole Blood

Jones, A.R., & Frazier, S.K. (2014). Increased Mortality in Adult Trauma Patients Transfused with Blood Components Compared with Whole Blood. *J Trauma Nurs; Jan-Feb 21*(1): 22-29.

This work was supported in part by a Centre grant to the University of Kentucky College of Nursing from NIH, NINR, 1P20NR010679. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Nursing Research or the National Institutes of Health.

Synopsis

Hemorrhage is a preventable cause of death among trauma patients, and management often includes transfusion, either whole blood or a combination of blood components (packed red blood cells, platelets, fresh frozen plasma). We used the 2009 National Trauma Data Bank to evaluate the relationship between transfusion type and mortality in adult major trauma patients (n = 1745). Logistic regression analysis identified three independent predictors of mortality: Injury Severity Score, emergency transfer time, and type of blood transfusion, whole blood or components. Transfusion of whole blood was associated with reduced mortality; thus, may provide superior survival outcomes in this population.

Introduction

Trauma is the leading cause of death for individuals aged 1-44 years.(1) Exsanguination is the primary cause of preventable death;(2-5) traumatic hemorrhage accounts for 35% of pre-hospital deaths, and more than 40% of deaths that occur within the first 24 hours after injury.(5) Transfusion of whole blood or blood components is the primary means of managing hemorrhagic shock that results from traumatic injury, in conjunction with effective control of bleeding. Yet, there is a lack of evidence to support the most appropriate type of trauma resuscitation transfusion, whole blood or blood components, which includes packed red blood cells (PRBCs), platelets (PLTs), and fresh frozen plasma (FFP).

Approximately 5 million individuals receive blood or component transfusions in the United States each year, with nearly 24 million units transfused annually.(6, 7) An average transfusion of PRBCs requires approximately three pints of donated blood, and a single trauma patient may require up to 100 units of PRBCs during resuscitation.(6) In 2011, whole blood transfusions accounted for only 0.15% of total transfusions; thus, blood components are the primary choice for transfusion.(8) In addition, 10.2% of all PRBC transfusions and 4.4% of all PLT transfusions were used by the Trauma/Emergency Department (ED) services.(8) The average cost of a unit of PRBCs was $225.42 and a unit of apheresis PLTs was $535.17.(8) Thus, transfusion consumes increasingly scarce financial resources and requires the continuing generosity of millions of donors annually.

An association between blood transfusion and greater mortality has been previously reported.(9, 10) Kapan and colleagues found that in patients who required

damage control surgery for trauma, transfusion volume was an independent predictor of mortality; those who died received 3 more units of blood than those who survived, in spite of similar injury severity score (ISS).(11) Cripps and colleagues found that in trauma patients who required activation of the massive transfusion protocol, those who died received 8 more units of PRCs, and the ratio of PRCs to FFP was also significantly greater (1.47/1.94).(12) However, those who died had a significantly higher admission ISS and a lower Glasgow coma score; thus, those who died were more severely injured. In a recent review of trauma transfusion practices over 6 years, Kutcher and colleagues identified a decrease in the volume of crystalloids infused, in conjunction with an attempt to emulate whole blood, using the combination of PRCs, FFP and platelet infusion; each reduction of 0.1 in the ratio of PRC/FFP was associated with a 6% reduction in mortality.(13) Thus, there is evidence to support the importance of not only the volume of transfusion, but also the type of blood transfused in trauma patient outcomes.

Researchers recently suggested that the transition from transfusion of whole blood to blood components during trauma resuscitation occurred without sufficient evidence to support this ubiquitous change in practice.(14) The transition to component transfusion occurred as longer storage times were achieved, and was intended to enhance the efficient use of a scarce resource, blood. Spinella and colleagues advocated for the use of whole blood transfusion after observing that transfusion of warm, fresh whole blood for treatment of hemorrhagic shock was associated with improved survival at both 24 hours (whole blood - 96%, components - 88%, $p = 0.018$) and 30 days (whole blood - 95% components - 82%, $p = 0.002$), when compared to those transfused with multiple blood components.(15) Improved survival with whole blood transfusion was attributed to lack

of anticoagulants and additives that are inherent to stored blood components.(15) Similarly, Nessen and colleagues found that infusion of fresh whole blood was an independent predictor of survival in those injured in combat (~ 90% reduction in likelihood of mortality), when compared with those who received multiple blood components, in spite of higher ISS, and lower admission blood pressure and core body temperature.(16)

Although management of hemorrhage focuses on control of blood loss and replacement of circulating volume, best practices related to transfusion have yet to be established. Thus, the aim of this study was to examine the association of type of blood transfusion, whole blood or blood components, with mortality in adult major trauma patients. We hypothesized that those who received whole blood would have a decreased likelihood of mortality compared to those who were transfused a combination of blood components in the management of their hemorrhage after trauma.

Methods

We performed a secondary data analysis of the 2009 National Trauma Data Bank (NTDB) data set.(17) Five hundred sixty-seven facilities from across the United States voluntarily submitted data from all trauma patient admissions in 2008 to form the database (n = 627,664).(18) All data were de-identified prior to distribution of the data set.

Sample

We included those patients who were aged 18-45 years, had ISS greater than 25 indicating critical injury, were admitted to the hospital after care in the ED, and who received blood transfusion, either whole blood or blood components, as part of their

emergency care. Patients were excluded from the analyses when they were dead on arrival to the emergency department, or were discharged to home after ED care and not admitted to the hospital.

Measures

Sociodemographic Variables

Sociodemographic variables included in the analyses were age, gender, and ethnicity. Due to small numbers of minorities in the database, ethnicity was classified as Caucasian or other. Older age (≥ 55 years of age) is a risk factor for mortality in trauma victims because of pre-existing comorbidities, and decreased physiological response to injury.(19) Thus, we excluded those over age 45 years of age. We controlled gender in our analyses, as there is evidence to support gender differences in trauma survival and recovery, although this issue continues to be debated.(20-24)

Injury Severity

The ISS is a measure of trauma severity.(25) Scores are based on the extent of physiologic damage in the three most severely injured areas of the body (head/neck, face, chest, abdominal/pelvic, extremities/pelvic girdle, external) using the Abbreviated Injury Scale.(25) These scores are then squared and summed to achieve the ISS. Totals for the ISS range from to zero to 75, with higher scores indicating more severe injury. A typical cutpoint used in the literature to identify more severely injured patients is an ISS of 15 or greater, with a score of 25 indicating a critical state of injury.(26-30) The NTDB reports three versions of the ISS: the raw score reported by the hospital attending physicians, the score calculated in the database from the reported AIS scores, and the score that is

calculated from International Classification of Diseases, Ninth Revision (ICD-9) codes.(31)

For this analysis, the ISS calculated from the ICD-9 codes was used as this was deemed the most valid for comparison purposes. The ISS is positively, although not linearly, correlated to mortality. Although higher ISS scores translate to higher likelihood of mortality for the trauma patient, given the scoring system of the ISS based on AIS scores, it is possible to have greater mortality rates for lower ISS scores (i.e. higher mortality for patients with an ISS of 16 versus 17) due to the physiologic location of the injury.(25, 32) The ISS has equivocal evidence to support the reliability and validity(33, 34) of the measure, and there are few evaluations of the psychometric properties. However, this measure continues to be widely used in trauma practice, and it is an accepted standard for injury severity measurement.(32, 35)

Emergency Medical System (EMS) Transfer Time

EMS transfer time was defined as the time from dispatch of emergency medical services to the time the patient arrived at the ED measured in minutes. We controlled the EMS transfer time because there is evidence that prolonged time to definitive treatment is associated with greater blood loss, hypothermia, and acidosis, thereby increasing risk for mortality.(36)

Transfer from Another Facility

Patients were dichotomized into those admitted directly to the ED from EMS transfer and those transferred to a trauma center ED from another clinical facility. We controlled for this variables, as this may produce delay in definitive treatment.(37)

Blood Product Administration

Blood products were categorized as administration of PRBCs and PLTs in combination, or whole blood transfusion (WBT). Data were not available for transfusion of fresh frozen plasma; thus, this component was not included in this analysis.

Mortality

Mortality was defined as death during the hospital stay for trauma, and was determined and reported by the attending physician.

Statistical Analyses

Sample characteristics were analyzed using descriptive statistics, independent t-tests and χ^2 as appropriate to describe the entire sample, and to compare those receiving blood components (PRBCs, PLTs, PRBCs/PLTs) with those who received whole blood, respectively. We used logistic regression to test the hypothesis that those transfused with whole blood would have a decreased likelihood of mortality compared to patients transfused with blood components after controlling for age, gender, and ISS. Demographic variables age, gender, and ISS were entered into the first regression block; EMS transfer time and transfer of the patient from another facility were entered into the second block. The blood product transfusion type, whole blood or components, was entered into the third block. An alpha level of 0.05 was set a priori to determine significance, and all analyses were performed using PASW, release 20.0 (SPSS, Inc., Chicago, IL).

Results

Characteristics of the Participants

Participants (n = 1745) included in this analysis were primarily male (72%), Caucasians (63%), aged 29 ± 8 years with an Injury Severity Score of 35 ± 13 indicating critical injury (Table 1.).(26-29) Heart rate (HR) and systolic blood pressure (SBP) were available from both the EMS and emergency department records. Participants had a mean EMS HR of 99 ± 26 and a mean emergency department HR of 101 ± 26. Mean EMS SBP for the sample was 122 ± 28, with a mean emergency department SBP of 127 ± 30. The average EMS transfer time for these patients was 19 ± 16 minutes, with a median time of 14 minutes. Only 21% of patients were transferred from another facility. Twenty-six percent of these patients died during hospitalization for their traumatic injuries. Ninety-five percent of participants received blood components (n = 1,662); only 5% received whole blood (n = 83); this demonstrated the ubiquitous nature of component transfusion.

Those receiving whole blood and those receiving blood components were compared with independent t tests or χ^2 analyses based on the level of measurement (Table 1). Patients who received whole blood were 2 years younger than those who received components (whole blood - 27 ± 8 years, components - 29 ± 8 years, p = 0.01), and the proportion of females who received blood components was significantly greater than the proportion who received whole blood (components - 29%, whole blood -17%, p = 0.02). While a statistically significant difference in mean SBP was detected among the transfusion groups when measured in the emergency department, this difference was not clinically significant (whole blood – 112 ± 29 mmHg, components – 120 ± 32 mmHg, p = 0.036). There were no other differences between the groups.

Mortality Predictors

We used logistic regression to determine independent predictors of mortality (Table 2). The model fit was evaluated using the Omnibus Tests of Model Coefficients and the Hosmer-Lemeshow test; these analyses determined that we identified a significant model ($p < 0.001$) with acceptable model fit ($p = 0.318$), respectively. Data were entered into the regression in blocks to control potential confounding variables and evaluate their relationship to mortality.

The regression revealed three independent predictors of mortality: the ISS, EMS transfer time and type of transfusion, whole blood or components (Table 2). With each one-unit increase in ISS, patients were 14% more likely to die (OR 1.014, 95% CI 1.005 – 1.024, $p = 0.004$), and for each minute increase in EMS time, patients experienced a 1.2% decrease in likelihood of mortality (OR 0.987, 95% CI 0.975 – 0.999, $p = 0.035$). After controlling for age, gender, EMS transfer time and transfer from another facility, those patients who were transfused with blood components were 3.2 times more likely to die when compared with those who received whole blood (OR 3.164, 95% CI 1.314 – 7.618, $p = 0.010$). Thus, our hypothesis was supported by the analysis.

Discussion

We found that in this large sample of adult trauma patients, the type of transfusion, whole blood or blood component, was an independent predictor of mortality; those patients who received blood component transfusion were 3 times more likely to die when compared with those who received whole blood transfusion, even though ISS was identical. Other independent predictors of mortality were the ISS and the EMS transfer

time. Mortality risk increased 14% for each one unit increase in ISS, and decreased by 1% for each additional minute of EMS transfer time.

Similar to our finding, other investigators have found that transfusion of whole blood produced superior survival compared to component transfusion. Combat patients who received whole blood had twice the likelihood of 30-day survival compared to those receiving blood components (OR 2.15, 95% CI 1.21-3.8, p = 0.016). Seghatchian and Samama concluded that fresh whole blood was superior to stored component transfusion with a 1:1:1 ratio (PRBCs:FFP:PLTs) in the prevention of coagulopathy in trauma patients, and Makley and colleagues found that transfusion of whole blood averted an inflammatory response produced by crystalloid resuscitation in animals after trauma.(38, 39) In contrast, Ho and Leonard found no difference in 30-day mortality in patients who received whole blood compared with components for massive transfusion, defined as $\geq$ 10 units; however, only one fourth of these patients were treated for traumatic injuries, patients were older than ours (mean age 52 $\pm$ 20 years), and diagnoses included gastrointestinal bleeding, cardiothoracic surgery and other types of surgery.(40) Thus, pre-existing comorbidities may have produced a confounding effect on mortality.

There is evidence that the age of blood components may have a significant effect on survival after transfusion. Current blood bank practices include the rotation of older PRBCs to trauma centers with high patient volume to reduce waste, as these centers are more likely to transfuse these components before they reach expiration and must be discarded.(41) Although the storage life of PRBCs is 42 days, there are predictable and identifiable morphological, biochemical, and functional alterations that occur and

produce a “storage lesion”; the older the age of stored components, the greater the changes.

Morphological alterations found with storage lesion include change from the normal smooth, deformable disc-shape erythrocytes that easily bend to flow through the microcirculation, to a spheroechinocyte, a sphere-shaped cell with protrusions, which is rigid and more likely to adhere to the endothelium of the microcirculation.(42, 43) Release of submicron-sized fragments of the cellular membrane and hemoglobin, known as microparticles, is also a component of the storage lesion.(44) These microparticles stimulate inflammation, have procoagulant activity, and contain hemoglobin, which is a scavenger of nitric oxide, an endothelium-derived relaxing factor.

Biochemical alterations found with the storage lesion are associated with continued cellular metabolism after donation, and include reduction in 2,3 diphosphoglycerate (2,3 DPG),(45) which is important in the release of oxygen from the hemoglobin molecule, decreased cellular pH,(46) increased lactate (46) and intracellular potassium,(47) increased release of ubiquitin, an immune modulating protein,(48) and collection of lipids, cytokines and free iron released from hemolyzed cells.(46) The global consequences of the storage lesion after transfusion are rapid destruction of spheroechinocytes, reduced microcirculatory blood flow, altered coagulation, reduction in tissue oxygen delivery, ineffective endothelial vasoregulation,(45) impaired immune response, and systemic inflammation.

There are also significant differences in 24-hour survival of erythrocytes after transfusion; packed cells stored for 25-35 days demonstrated double the degree of hemolysis when compared with those stored for less than 10 days (11% versus 22%, $p \leq$

0.05).(49) Unfortunately, we were unable to determine whether the storage lesion in the infused PRBCs was an independent predictor of mortality, as age of the transfused products was not available in the data set. This is an important focus for continued research.

We also found that the ISS was an independent predictor of mortality. This is not surprising, and provides additional evidence for the construct validity of the ISS. Numerous other investigators have also found the ISS to be a predictor not only for mortality, but also adverse outcomes and complications in the trauma population.(50-56) Dutton, Lefering, and Lynn found higher ISS to be predictive of both an increased risk for transfusion and an increased volume of transfused blood or blood components.(57) Similar findings were reported in a systematic review performed, indicating injury severity as an important predictor of the need for transfusion in the trauma population.(58) As those included in our analysis had the statistically same ISS, the need for transfusion should have been statistically equivalent based on this evidence.

The third independent predictor of mortality was the EMS transfer time with each additional minute of transfer time associated with a 1% decrease in mortality likelihood. The impact of transport time on the likelihood of mortality is equivocal, with data supporting increased and decreased likelihood, as well as no impact on mortality. Longer transport times are often associated with pre-hospital interventions. Recent evidence from the Prospective Observational Multicenter Massive Transfusion (PROMMT) group identified a 16% reduction in the likelihood of mortality in trauma patients who received intravenous fluid administration (59); Bernard and colleagues found that rapid sequence intubation in the pre-hospital setting was associated with

improved functional outcome in adults patients with severe traumatic brain injury.(60) Thus, pre-hospital interventions that require time have been shown to improve survival. We consider this to be a viable hypothesis for our finding.

In contrast, Gonzalez and colleagues found that longer EMS transport times were associated with higher mortality in rural trauma patients.(36) Johnson and colleagues identified a significant survival benefit in those trauma patients transported by private vehicle compared with EMS transport after controlling for ISS; thus, shorter transport time predicted greater likelihood of survival.(61) However, McCoy and colleagues found no association between transport time and mortality in nearly 20,000 patients with blunt and penetrating trauma(62), and The Resuscitation Outcomes Consortium Investigators also found no association between EMS activation time, on-scene, transport or total EMS time and mortality.(63) Thus, this variable is more complex than just the time in minutes and requires more systematic investigation, with consideration of the pre-hospital care administered.

Our study was limited in several ways. First, the NTDB dataset provided retrospective data and contains limited variables for analysis. For example, transfusion of blood components or whole blood is available as a dichotomous yes-no variable, but quantification of the units of each component or units of whole blood transfused was not available. Furthermore, while EMS and ED heart rate and systolic blood pressure were available for analysis, these variables are not supported by prior research evidence as independent predictors of transfusion requirement or as adequate indicators of hemorrhage status; thus, they were not included in the regression.(64, 65) In addition, the validity of the data could not be evaluated and the accuracy of data entry is uncertain.

Errors in the database are possible because of the multiple hospitals, institutions, and data entry personnel who contributed to the database. We studied only a subset of younger patients in our analyses; thus, older patients may have different responses.

Conclusion

We found that transfusion of whole blood rather than blood components produced superior survival in adult trauma patients from the NTDB. The inclusion of a geographically diverse sample increases the generalizability of our findings. The current practice of ubiquitous component administration in the trauma population requires further study to ensure that optimal trauma outcomes are achieved in those who receive transfusion. Our findings also support the construct validity of the ISS, a common instrument, used in clinical practice and research to quantify severity of injury. Unfortunately, our transport time finding adds to the ambiguity about mortality and pre-hospital care, and demonstrates the need for further systematic evaluation of this complex issue.

Table 4.1 Characteristics of the Participants (n = 1745)

Variable	Total Sample n = 1745	Whole blood n = 83	Blood components n = 1662	P value
Age in years	29 ± 8	27 ± 8	29 ± 8	0.01
Gender Male	1253 (72%)	69 (83%)	1184 (71%)	0.02
Ethnicity Caucasian	1105 (63%)	47 (57%)	1058 (64%)	0.20
Injury Severity Score	35 ± 13	39 ± 17	35 ±13	0.13
EMS* Transfer Time in minutes	19 ± 16	15 ± 11	19 ± 16	0.15
Transferred from Other Facility (Yes)	367 (21%)	15 (18%)	352 (21%)	0.50
Mortality	446 (26%)	17 (21%)	429 (26%)	0.27

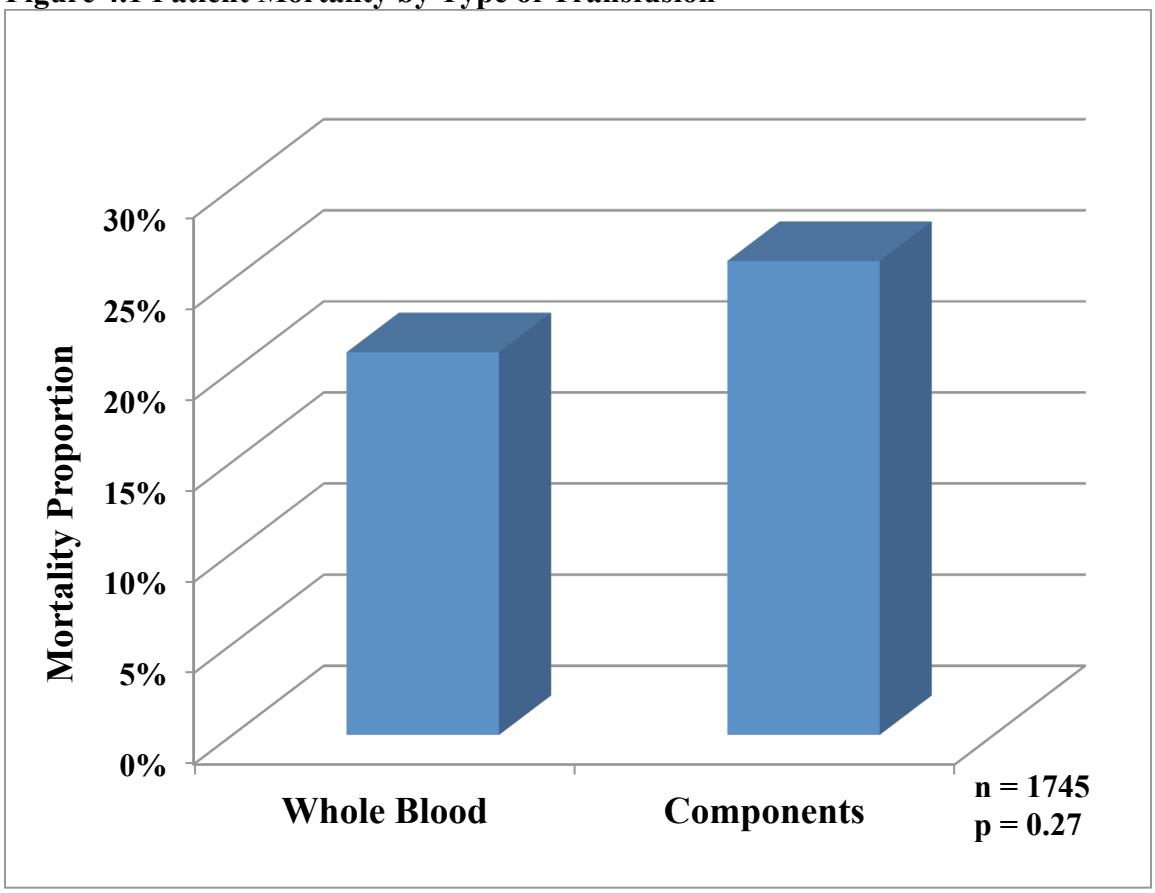
Values are mean ± standard deviation or frequency (proportion)
Groups compared using independent t tests for continuous variables and Chi square or Fishers Exact test for categorical variables
* Emergency Medical Services

Table 4.2 Independent Predictors of Mortality In Adult Trauma Patients (n = 1745)

Variable (Reference group)	β	Exp β	95% CI	P value
Age	-.009	.991	.975 – 1.008	0.32
Gender (Male)	-.274	.760	.554 – 1.044	0.09
Injury Severity Score	.014	1.014	1.005 – 1.024	0.004
EMS* Time	-.013	.987	.975 – 0.999	0.035
Transfer (No)	-.124	.883	.569 – 1.370	0.58
Blood Product (Whole)	1.152	3.164	1.314 – 7.618	0.01

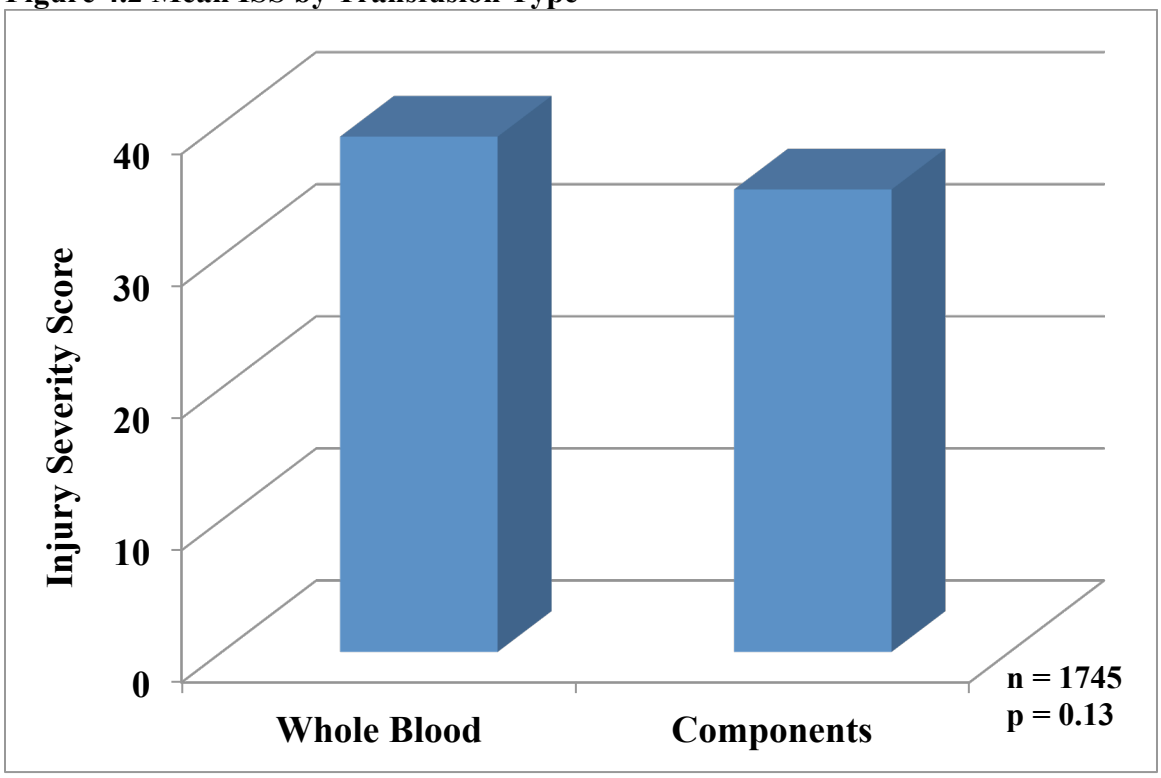
Logistic regression analysis
For categorical variables, comparison group in parentheses
* Emergency Medical Services

Figure 4.1 Patient Mortality by Type of Transfusion



Proportions compared with Chi Square analysis

Figure 4.2 Mean ISS by Transfusion Type



Scores compared using independent t tests

Figure Legend

Figure 4.1 Patient Mortality by Type of Transfusion

Figure 4.2 Mean ISS by Transfusion Type

CHAPTER FIVE

Predictors of Inflammatory Complications in Patients Who Received Component Transfusion After Trauma

Synopsis

Transfusion of blood components is associated with increased risk of in-hospital complications and mortality. Patients experience a decrease in physiologic reserve paired with release of anti-inflammatory and proinflammatory mediators following traumatic injury, making them subject to potential development of inflammatory complications. Findings from a secondary analysis of the Inflammation and Host Response to Injury Trauma-Related Database are presented (n = 1,656). Findings revealed that the total transfused volume of packed red blood cells in the first 24 hours following hospital admission was associated with development of inflammatory complications, as was the presence of comorbidities and injury severity.

Introduction

Five million individuals die from trauma annually worldwide according the World Health Organization;(1) traumatic hemorrhage accounts for 35% of pre-hospital deaths, and more than 40% of deaths within the first 24 hours after injury.(2) Trauma induces physiological mechanisms intended to halt blood loss. However, these mechanisms may be ineffective; continued bleeding will ultimately lead to hemorrhagic shock, "a failure of adequate tissue perfusion due to lack of circulating blood volume".(3) A recent investigation demonstrated that hemorrhagic shock associated with trauma resulted in microcirculation alterations for up to 72 hours after restoration of adequate circulating volume, as a consequence of tissue hypoperfusion, inflammation, and resuscitation strategies.(4) These strategies included crystalloid infusion and transfusion of blood or blood components. However, blood transfusion volume and ratio of components in the transfusion (i.e. packed red blood cells [PRBCs] to fresh frozen plasma [FFP] to platelets [PLTs]) are independently associated with mortality;(5, 6) in fact, administration of blood components was associated with triple the likelihood of mortality compared with those who received whole blood (Odds ratio [OR} 3.164, Confidence interval [CI] 1.314 – 7.618, $p = 0.01$) in civilian patients after trauma.(7)

There is a plethora of evidence to support an association between transfusion of blood components with an increased risk of complications (anywhere from a 37% (8) to over 3400%) (9) and mortality (ranging from 5% (10) to almost 80% (8)).(8-10) Although still controversial, some investigators concluded that there is a survival benefit when receiving blood components in a 1:1:1 ratio of PRBCs, FFP, and PLTs. This ratio is thought to mimic the composition of whole blood, which has been associated with

improved survival in high volume transfusion.(11-18) In addition, Kutcher and colleagues found that a reduction in volume of infused crystalloid, in conjunction with emulation of whole blood using a ratio of 1:1:1 for PRBCs, FFP and PLTs infusion, produced a 6% decrease in mortality for each reduction of 0.1 in the ratio.(19)

Current standard of practice for blood banks includes rotation of older stored components to trauma centers to reduce waste, as there are established storage times after which these components must be discarded.(20) However, stored components (PRBCs and PLTs) experience a storage lesion, which results in morphological, functional, and biochemical alterations of erythrocytes and platelets.(21, 22) The global consequences of the storage lesion are rapid destruction of abnormal erythrocytes known as spheroechinocytes, reduced microcirculatory blood flow, coagulopathy, reduction in tissue oxygen delivery, ineffective endothelial vasoregulation, impaired immune response, and systemic inflammation.(23-26)

The physiological response to traumatic injury includes the release of proinflammatory cytokines (e.g. tumor necrosis factor-∝ [TNF-∝], interleukin-6 [IL-6]) in reaction to injury to tissues, and anti-inflammatory cytokines to balance the proinflammatory cytokines, and return the body to a state of homeostasis.(27) The systemic inflammatory response to trauma is the result of damage-associated molecular patterns, which are secreted by activated immune cells that include neutrophils.(28) Cellular destruction and release of mitochondria and cellular peptides stimulate a particularly rigorous immune reaction, as they are interpreted to be foreign molecules.

Current published theories propose that release of proinflammatory cytokines in the phase immediately following traumatic injury may be associated with detrimental

clinical outcomes.(28) In patients with severe traumatic injury, especially those receiving transfusions of blood or blood components, inflammation may become exacerbated and prolonged. Xiao and colleagues (29) examined leukocyte gene expression in patients following trauma, and found that expression was up-regulated for cytokines including IL-6 and IL-10, and down-regulated for cytokines such as T cell regulators, beginning within 12 hours of trauma and remaining throughout 28 days following injury.

In the same study, investigators also compared genomic expression patterns of whole blood leukocytes for patients who experienced a complicated (development of organ failure and/or infection in addition to recovery > 14 days, no recovery, or death) versus uncomplicated (recovery < 5 days) recovery.(29) Their findings revealed that patients with an uncomplicated recovery experienced a return to baseline in their leukocyte gene expression within 2 weeks of injury; in patients with complicated and/or prolonged recovery, early leukocyte gene expression changes were greater, and changes observed immediately following injury had not returned to baseline by 28 days post-injury. Thus, genomic expression and release of proinflammatory and anti-inflammatory cytokines occurs in the first 24 hours post-injury, and increased expression is prolonged in those with more complicated recovery, including the development of inflammatory complications during hospitalization (e.g. acute respiratory distress syndrome, acute renal failure, nosocomial infections, surgical site infections, sepsis, and ventilator-associated pneumonia).

Infection after trauma is common. Investigators found that 52% of trauma patients developed an infection while in critical care; those who presented to the emergency department (ED) with shock (defined using base deficit > 12 mEq/L) developed

infectious complications more rapidly compared to those with hemodynamic stability (median 3.5 days versus 5 days, $p = 0.01$).(30) Other investigators found infectious complications were associated with a prolonged length of stay in patients after trauma, ranging from a median of 13 days for urinary tract infection/acute cystitis (Interquartile Range [IQR] 7-24 days) to a median of 27 days for both sepsis (IQR 17-41) and surgical infections (IQR 17-41).(31) These same investigators found a significant increase in the relative risk for mortality associated with complications, including cardiovascular events (33%), renal failure (24%), ARDS (16%), and sepsis (13%) ($p \leq 0.05$).(31) Recently, median hospital charges for patients after trauma with an infectious or incisional complication were 70% greater than the costs for those without these complications (with infection - median $171,376 (IQR $83,980 – $310,104); without infection - median $50,980 ($26,398 – $94,631), ($p < 0.001$).(32)

Thus, inflammatory and infectious complications increased not only patient length of stay and likelihood of mortality, but also costs for both patient and hospital. Furthermore, transfusion of blood components is associated with additional inflammatory activation and increased complications and mortality.(9, 33) Therefore, the purpose of this study was to evaluate the relationship of transfusion-related variables (volume and ratio of transfused components) to development of inflammatory complications in trauma patients.

The aims of this study were: 1) to evaluate the prevalence of inflammatory complications (organ failure, nosocomial infections, Acute Respiratory Distress Syndrome [ARDS], pneumonia, central line associated blood stream infection [CLABSI], urinary tract infection [UTI], sepsis or septic shock) developed during hospitalization in

an adult major trauma population; 2) to determine whether blood transfusion volume and ratio of components transfused within the first 24 hours following admission to the ED predicted development of inflammatory complications during hospitalization; 3) to determine whether blood transfusion volume and ratio of components transfused within the first 24 hours following admission to the ED predicted time to diagnosis of inflammatory complications; 4) to determine whether inflammatory markers measured within the first 24 hours following admission to the ED and receipt of blood transfusion predicted development of inflammatory complications during hospitalization.

Methods

We performed a secondary analysis using the Inflammation and Host Response to Injury Glue Grant trauma database (TRDB).(34) The data available through the TRDB contained de-identified human data prospectively collected from eight Level I trauma institutions across the United States. This dataset included data from over 1,600 patients after blunt trauma hospitalization (up to 28 days), who were enrolled between 2003 to 2009. Vital signs available for analysis included temperature, heart rate, mean arterial pressure, and respiratory rate. The values were recorded as the highest and lowest values in association with calculation of the Acute Physiology and Chronic Health Evaluation (APACHE) II score, which is based on assessment of vital signs and laboratory values in the first 24 hours of hospitalization. This instrument is used to quantify severity of illness in critical patients, and to estimate requirements for hospital resources, determine nursing staffing patterns, and predict mortality.(35) As the NISS is a more widely accepted and used instrument for categorizing trauma patients, APACHE II scores were not included in the analysis.

Transfusion data were recorded at 6-hour intervals beginning from the time of ED admission. Pre-hospital crystalloids and blood volume received prior to patient admission to a tertiary care center were collected as well. Demographic variables age, sex, and ethnicity were collected on patient admission to the ED. The New Injury Severity Score was computed after the patient was admitted to the hospital, and was based on injuries the patient sustained.(36)

Sample and setting

We included patients between 18 and 65 years of age, who received transfusion of blood components within the first 24 hours following injury, and with an Injury Severity Score (ISS) of $\geq$ 15, which indicated severe injury. We excluded participants above the age of 65 due to likelihood of multiple comorbidities, and decreased physiologic reserve that may have limited survival.(37) Participants were also excluded if they died within the 24 hours following admission to reduce survival bias. A small subset of patients was recruited by the TRDB investigators for evaluation of inflammatory biomarkers following admission in addition to collection of other clinical variables.

We determined that a logistic regression would have at least 80% power to detect an odds ratio of 1.5 when the sample size was 250, with the assumption that the rate of complications at the mean of the explanatory variable was 50%, and that there was moderate correlation among the variables included in the model. After exclusion criteria were applied, our sample included 1,656 patients. We then further selected those who received transfusion of all three major components (PRBCs, PLTs, and FFP) in the first 24 hours.

All data were collected from Level I civilian trauma centers. The Level I designation is a reflection of the verification provided by the American College of Surgeons that denoted the ability of a facility to provide appropriate care for the most severely injured patients around the clock.(38, 39) Level I facilities typically serve large cities or metropolitan areas, and may provide higher level services to smaller hospitals in the surrounding areas as well, with provision for the transfer of critically injured patients to a higher level of care when necessary.(39)

Measures

Sociodemographic Variables

Sociodemographic variables included in our analyses were age, sex, and ethnicity. Ethnicity was classified as Caucasian, African American, or other, due to small numbers of minorities other than African American. Data related to the hospital location consisted of de-identified site codes, thus limiting our ability to specify the region of the country in which the patient was treated. No information was available regarding education, pre-existing health conditions, insurance status, socioeconomic status, or employment.

Clinical Variables

Twenty-eight day survival was recorded as a dichotomous yes/no variable. Hospital length of stay in days was also recorded as full days. For those who survived beyond 28-days, the total hospital length of stay was documented. Vital signs were analyzed highest and lowest as recorded during the first 24 hours of hospitalization. Temperature was originally recorded in degrees Celsius then converted into degrees Fahrenheit. Glasgow Coma Score (GCS) total values were recorded in two ways, as the worst GCS experienced during the first 24 hours of hospitalization, and GCS while in the

ED. Laboratory values were also available to characterize the sample. Values recorded as part of the APACHE II data included hematocrit and arterial pH, again recorded as the highest and lowest values. Other laboratory values measured while the patient was in the ED were serum lactate, hemoglobin, international normalized ratio (INR), and base deficit. The volume of crystalloids infused prior to admission to the ED was also recorded and measured in milliliters (mL).

Injury-Related Variables

Mechanism. Mechanism of injury was originally categorized as one of the following: fall, machinery, motor vehicle collision (MVC) – occupant, MVC – motorcyclist, MVC – cyclist, MVC – pedestrian, struck by or against, or other. Due to small numbers of those in categories of falls, machinery accidents, cyclists, pedestrian, and struck by or against, these patients were then grouped in the other category.

Injury Severity Score. The Injury Severity Score (ISS) is based on the Abbreviated Injury Scale (AIS), an instrument used to assign scores to the areas of the body most affected by injury.(40) The AIS is an ordinal scale with values assigned to each area of the body based on the most severe injury to that area; values ranged from 0 indicating no injury, to 6 indicating a fatal injury.(40, 41) The ISS is calculated by taking the three areas of the body most severely injured according to corresponding high AIS scores, squaring them, and summing them. ISS values range from 0 to 75, with higher numbers indicating more severe injury. Evidence supporting the reliability and validity of the ISS is equivocal.(42) Nonetheless, the ISS is commonly used in trauma centers and research studies to quantify the extent of physiologic injury.(43)

The version of the ISS found in the TRDB, the New ISS (NISS),was a modified version of the original instrument. The NISS was released in 1997, and has been shown to be a better predictor of mortality compared with the ISS.(36) The NISS uses the same range of scores as the original ISS, but the instrument evaluates the three most severe injuries sustained by the patient, regardless of the area of the body in which the injuries are found.(36) For our study, we included patients with a NISS score of 15 or higher, a cut point commonly used in trauma research to distinguish those with severe and critical injuries.(44-46) For analysis of specific aims 2-3, NISS was transformed into a dichotomous variable, with a score of $\geq$ 25 indicating severe/critical injury, and a score of $\leq$ 24 indicating mild/moderate injury.

Comorbid Burden

Comorbidities were indicated in the database with yes/no responses for each specific comorbid condition. Total number of comorbidities was calculated by summing the number of affirmative responses. Values ranged from 0 – 33. Comorbidities evaluated by the investigators included: hypertension which required medications, myocardial infarction, congestive heart failure, atrial and ventricular tachyarrhythmias, peripheral vascular disease, cerebrovascular disease, dementia, seizure disorder, previous traumatic brain injury, hemi- or paraplegia, Parkinson's disease, chronic obstructive pulmonary disease, rheumatologic disease, peptic ulcer disease, liver disease, diabetes, hypo- or hyperthyroidism, chronic renal dysfunction, history of malignancy, metastatic solid tumor, human immunodeficiency virus, acquired immunodeficiency syndrome, congenital or acquired coagulopathy, smoking, chronic alcoholism, intravenous drug use, homelessness, psychiatric disorder, solid organ transplant recipient, and chemotherapy or

radiation therapy in the last 30 days. Patients were dichotomized into those who had at least one comorbidity versus those who had none. With this variable, we controlled for comorbid burden in the analyses, as more complications and reduced survival are associated with a greater comorbid burden.(47)

Transfusion-Related Variables

Total volume of blood components transfused included the total number of mL transfused in the first 24 hours after trauma. Total volume of PRBCs, FFP, and PLTs were available for analysis. PRBC volume was converted from mL to units based on the assumption that the average volume per unit of PRBCs is 300 mL.(48) FFP volume was converted to units using an average unit volume of 200 mL,(49) and PLT volume was converted based on an average volume of 50 mL.(50) Ratios of components were separated into the ratio of PRBC:FFP and PRBC:PLT, again calculated based on transfusion volume in the first 24 hours after admission, and presented in decimal notation (instead of 3:4, recorded as 0.75 or 0.8). For logistic regression and time-dependent analysis, the transformed ratio values were categorized as those between 0.5-1.5 and those outside of that range. We performed this transformation to determine the impact of the ratio moving away from 1:1 (absolute value of 1.0), as evidence exists to support a ratio of 1:1 as beneficial to survival and development of complications for trauma patients receiving component transfusion.(51)

Inflammation

Complications

Complications chosen for analysis included organ failure, ventilator-associated pneumonia (VAP), sepsis or bloodstream infection, catheter-related bloodstream

infection (CRBSI), urinary tract infection (UTI), acute respiratory distress syndrome (ARDS), and nosocomial infections (NIs). NIs included non-ventilator associated pneumonia, meningitis, sinusitis, endocarditis, acute cholecystitis, empyema, and pseudomembranous colitis. Diagnosis was based on pre-established criteria developed by the Glue Grant investigators.(52) Organ failure was based on the Denver score,(53, 54) where a total score of three or more indicated multiple organ failure. A score of one indicated presence of organ failure (pulmonary, cardiac, renal, or hepatic), but specific information pertaining to the failing organ was not available; thus, patients with a score of one or more were determined to have organ failure and were compared to those with a score of zero (no organ failure). Development of any inflammatory complications during hospitalization was categorized as a dichotomous variable in the dataset. Patients were considered to have developed inflammatory complications if at least one of the complications was diagnosed. Total number of complications was also calculated for those complications included in the analysis; values ranged from 0-7. Time to development of complication was calculated in the database as time from injury to time of diagnosis of a complication, measured in days. For patients with more than one complication during hospitalization, we used the time to first diagnosed complication.

Statistical Analyses

Initial Kolmogorov-Smirnov tests were used to determine the distribution of the data. Descriptive statistics including frequencies and percentages, medians (IQR), and means (standard deviations) were used to characterize the participants. Correlations of variables were evaluated to determine the presence of collinearity prior to logistic and

Cox regressions. An α of .05 was set a priori to determine significance. All analyses were performed using PASW, release 22.0 (SPSS, Inc., Chicago, Illinois).

Specific aim 1 was to evaluate the prevalence of inflammatory complications (organ failure, NI, ARDS, VAP, CLABSI, UTI, or sepsis) developed during hospitalization in an adult major trauma population. Frequencies were used to analyze the prevalence of these outcomes among patients.

The second aim was to determine whether blood transfusion volume and ratio of components transfused within the first 24 hours following admission to the ED predicted development of inflammatory complications during hospitalization. We used a logistic regression with the dichotomous variable created to indicate those who developed a complication and those who did not. Age, gender, and presence of at least one comorbidity were entered into the first block; NISS (categorical) was entered into the second block, followed by transfusion variables in the third block (24-hour total units of PRBCs, 24-hour total units of PLTs, PRBC:PLT ratio [categorical], and PRBC:FFP ratio [categorical). The 24-hour FFP total units were not included in the model, as this component does not develop the storage lesion. Investigators have suggested a protective mechanism women compared to men who have traumatic injury; thus, we controlled for gender in our analyses.(55-57)

To determine whether blood transfusion volume and ratio of components transfused within the first 24 hours following admission to the ED predicted time to diagnosis of inflammatory complications, a Cox proportional hazards model was used. Kaplan-Meier analysis and log-rank testing were used to compare time to complication for patients based on their ratio of PRBC:PLT units (0.5-1.5 versus other) and PRBC:FFP

units (0.5-1.5 versus other). For the Cox model, age, gender, and presence of at least one comorbidity were entered into the first block; NISS (categorical) was entered into the second block, followed by transfusion variables in the third block (24-hour total units of PRBCs, 24-hour total units of PLTs, PRBC:PLT ratio [categorical] and PRBC:FFP ratio [categorical]). The 24-hour FFP total units variable was again not included in the model.

The final aim was to determine whether inflammatory markers measured within the first 24 hours following admission to the ED and receipt of blood transfusion predict development of inflammatory complications during hospitalization. Due to the small number of patients with values available for the biomarkers (n = 17), these analyses were not performed.

Results

Characteristics of the Participants

A total of 1,538 patients were included in these analyses (Table 1.). The majority were Caucasian (90%), males (68%), with an average age of 39 $\pm$ 14. Patients had an average of 1 $\pm$ 1 comorbidity; one-third of patients had two or more comorbidities, and 39% had none. The three most commonly reported comorbidities were smoking (30%), chronic alcoholism (14%), and psychiatric disorders (11%). Patients were critically injured, with a median NISS of 39 $\pm$ 13; 70% were involved in a MVC as either an occupant of the vehicle, or as a motorcyclist. Approximately a quarter of patients (28%) were transferred from another hospital, with a 90% 28-day survival rate. GCS on admission to the ED was 8 $\pm$ 6; worst GCS recorded in the first 24 hours was 5 $\pm$ 4, which indicated significant loss of consciousness and cognitive impairment.

The lowest body temperature measured in the first 24 hours was an average of 95.0 ± 2.3 °F, whereas the average highest temperature in the first 24 hours was 100.4 ± 1.4 °F. Mean arterial pressure ranged from an average low of 52 ± 17 mmHg to an average high of 118 ± 19 mmHg in the first 24 hours of care. Heart rates were elevated, with an average low of 77± 21 beats/minute (bpm), and an average high of 137 ± 21 bpm. The average low pH for patients in the first 24 hours was 7.2 ± 0.1, with a mean first base deficit of -8.8 ± 4.4; the average high pH during the first 24 hours was 7.4 ± 0.1. On admission to the ED, the first median hemoglobin was 11.6 ± 2.6 grams/deciliter (g/dL), and the first mean INR was 1.4 ± 0.7. In addition, patients had an average low hematocrit of 24 ± 6% and a high of 39 ± 5%.

Prior to ED admission, patients received an average of 219 ± 592 mL of blood (not further specified) and a mean of 2,178 ± 2,236 mL of crystalloid. In the first 6 hours, 92% of these patients received PRBCs, 50% received FFP, and 26% received PLTs (Table 2.). By the end of the first 24 hours, all patients had received PRBCs, whereas 65% received FFP, and 40% received PLTs. Average volume of PRBCs transfused in the first 6 hours was 1,977 ± 2,329 mL or 6.6 ± 7.8 units. FFP infusion was an average of 705 ± 1,102 mL or 3.5 ± 5.5 units, with an average volume of PLTs infusion of 125 ± 330 mL or 2.5 ± 6.6 units. Ratio of PRBC:FFP units at 6 hours were a mean of 1.9 ± 1.9, meaning that patients received 1.9 units of PRBCs per unit of FFP. Mean PRBC:PLT ratio at 6 hours was 1.9 ± 2.1, which indicated that patients received nearly 2 units of PRBC per unit of PLTs. At 24 hours after admission, an average of 2,843 ± 3,599 mL PRBCs or 9.5 ± 12.0 units were transfused. Total FFP administered by 24-hours after admission equaled 1,195 ± 1,899 mL or 6.0 ± 9.5 units, and 242 ± 486 mL of PLTs or

2.5 ± 6.6 units were administered. Ratio of blood components for PRBC:FFP at the end of the first 24-hour period following admission were a mean of 1.9 ± 2.0 units. This indicated that patients received about 2 units of PRBC per unit of FFP. Mean ratio of PRBC:PLT was 2.6 ± 2.9. In other words, patients received about 2.5 units of PRBCs for each 1 unit of PLTs. A total of 376 patients received all three components at the end of the first 24-hour period following admission.

Thirty-two percent of patients were discharged home with or without services; almost one third of patients (27%) were sent to an inpatient rehabilitation facility, and another 24% went to a skilled nursing facility. Of those who died (10% 28-day mortality), major cause of death included brain death (25%) and multiple organ failure (19%).

Prevalence of Complications

Eighty-six percent of the sample developed at least one of the specified inflammatory complications (organ failure, VAP, CRBSI, UTI, sepsis, ARDS, or NI) (Table 3.). For those with organ failure, 76% had a score of one or greater and 36% developed multiple organ failure. Almost a third of patients developed VAP (27%). Only 3% of patients developed a CRBSI. Fourteen percent of patients developed a UTI, and 13% developed sepsis. Approximately a quarter of patients (24%) were diagnosed with ARDS. Almost half of the patients developed a nosocomial infection (45%). Time to diagnosis of first complication was a median of 5 days (IQR 2 – 8).

Inflammatory Complications Predictors

Logistic regression was used to determine independent predictors of inflammatory complication (Table 4.). Variables were entered by blocks to control for confounding and

determine relationships with development of inflammatory complications. Age, gender, and presence of at least one comorbidity were entered first, followed by NISS (categorical) and transfusion variables (24-hour total units of PRBCs, 24-hour total units of PLTs, PRBC:PLT ratio [categorical], and PRBC:FFP ratio [categorical). The Omnibus tests of model coefficients and the Hosmer-Lemeshow test were used to determine model fit; we developed a significant model ($p < 0.001$) with acceptable model fit ($p = 0.241$). We found two independent predictors of development of inflammatory complications, presence of comorbidities and the 24-hr total of PRBC units transfused. The presence of at least one comorbidity was associated with a 5-fold risk in development of inflammatory complications (OR 5.367, 95% CI 2.235-12.886, $p < 0.001$). With each additional unit of PRBCs transfused, risk of developing an inflammatory complication increased by 8% (OR 1.083, 95% CI 1.018 – 1.151, $p = 0.011$). Patient age was found to be protective for development of inflammatory complications, but was not statistically significant at the 0.05 level ($p = 0.053$).

Time to Complication Development

Cox proportional hazards model was used to determine predictors of time to development of inflammatory complications (Table 5.). Variables were entered into the model based on their potential for confounding and to evaluate their relationship with timing to development of inflammatory complications. Age, gender, and presence of at least one comorbidity were entered first, followed by NISS (categorical) and transfusion variables (24-hour total units of PRBCs, 24-hour total units of PLTs, PRBC:PLT ratio [categorical], and PRBC:FFP ratio [categorical). The Omnibus tests of model coefficients indicated that a significant model was identified ($p < 0.001$). Results of the Cox

regression revealed two independent predictors of time to complication development, NISS and 24-hr total PRBC units transfused. Those with severe or critical injury severity (NISS ≥ 25) experienced a 41% increase in the likelihood of developing an inflammatory complication compared to those with mild/moderate injury (NISS ≤ 24) (hazard ratio [HR] 1.409, 95% CI 1.034 – 1.920, p = 0.03). For each additional unit of PRBCs transfused, patients experienced a 1% increase in their likelihood of developing an inflammatory complication (HR 1.010, 95% CI 1.04 – 1.016, p = 0.001). A 20% increase in the risk of inflammatory complications was observed for those with male gender, but this was not statistically significant at the 0.05 level (p = 0.053).

Log-rank testing did not reveal any significant differences between either PRBC:FFP ratio groups or PRBC:PLT ratio groups in terms of time to development of complications (Figure 1.,2.). Median time to development of complications for the 24-hr PRBC:FFP ratio groups was 7 days for both ratio groups (p = 0.709). Median time to development of complications for those who received a 24-hr PRBCs:PLTs ratio between 0.5-1.5 was 8 days, compared to 6 days for those who were outside of this range (p = 0.282).

Discussion

A majority of patients transfused after traumatic injury (86%) developed at least one inflammatory complication during their hospitalization. Independent predictors of inflammatory complication were the presence of at least one comorbidity and the 24-hour total transfused volume of PRBC units. The 24-hour total of PRBCs transfused was also an independent predictor of time to development of inflammatory complications, as was

critical or severe injury as indicated by a NISS of 25 or more. Other transfusion-related variables were not predictive of time to complication development.

The 24-hr transfusion volume of PRBCs was an independent predictor of inflammatory complication development; thus the storage lesion in PRBCs must be considered as a potential mechanism. These effects include the eventual transition of cells to anaerobic metabolism and depletion of adenosinetriphosphate (ATP), a decrease in pH from lactic acid production and cellular edema with possible rupture due to failure of the ATP sodium-potassium pump within the cell membrane.(21) When cells experience a breakdown in membrane structure, the contents of the cell leak into the supernatant, or the preservative fluid. These contents include electrolytes, microparticles or pieces of phospholipid membrane, and proinflammatory mediators that circulate freely in the stored component.

The storage lesion worsens over time. The practice of transfusion of older stored components to patients with major trauma could result in worse outcomes.(20) However, data about the relationship between age of transfused PRBCs and adverse outcomes are equivocal.(58) Investigators suggested that patients who were injured severely enough to require transfusion of multiple units of PRBCs for resuscitation could experience exacerbated vasoconstriction after transfusion.(33) Thus, transfusion of older components would reduce circulation to tissues and lead to development of complications.(59) Others have suggested that over-transfusion of patients, or overloading them with components in order to restore circulating volume, resulted in worse outcomes, which would support our findings here.(33) Though the mechanisms that produced inflammatory complications

after trauma are not clear, our findings support this association. This requires further investigation.(8, 9)

The ratio of transfused components was not a predictor of inflammatory complications in this study. There is evidence to support transfusion of components in a 1:1:1 fashion for patients with major trauma who required 10 or more units of PRBCs in the first 24 hours.(60, 61) However, the optimal ratio for patients who require less transfused components has not been determined. Differences among patients and transfusion requirements may originate with the state of coagulation, where exsanguinating patients require more clotting factors through transfusion of FFP and PLTs compared to less severely injured patients.

The association between transfusion and inflammation in our patients supports the findings of other investigators. Cole and colleagues (30) analyzed a sample of 271 severely injured trauma patients (median ISS 29, IQR 21-36) with a median age of 35 (IQR 25-49) to determine rate of infection following critical care admission. These investigators found an overall an infection rate of 52%; those who developed infection received significantly greater volume of PRBCs in the first 24 hours of hospital care than those who did not develop an infection (median units 4, IQR 3.4-5.4, versus 3, IQR 2.1-3.7, $p = 0.01$). However, volume of PRBCs was not an independent predictor of infection in their analysis. These investigators also found a lower rate of infections than in our participants. This finding may be due to their smaller sample size or younger median age. Also, the investigators did not report the frequency of blood component transfusion among their sample, so it is possible that not all participants received transfusions, which could impact development of complications.

Other investigators evaluated the relationship between coagulopathy following trauma and development of infection during hospitalization.(62) They found that less than half of their sample developed an infection (45%). Of those who developed an infection, patients were older (median age 44 years, IQR 25-59 versus 32 years, IQR 24-42, $p = 0.02$) and more severely injured (median ISS 25, IQR 15-33 versus 10, IQR 5-18, $p < 0.01$) than those without infection. However, the investigators did not find any differences in coagulation between those who developed infection and those who did not, which supports the notion that the physical transfusion of components rather than the physiological need for transfusion based on presence of coagulopathy may be more intricately related to development of inflammatory complications.

The inflammatory response following trauma, in conjunction with transfusion of stored components with the storage lesion may play a role in the development of complications. One effect of the storage lesion is the leaking of intracellular contents including proinflammatory mediators into the supernatant or preservative fluid after breakdown in the phospholipid membrane. Transfusion of components containing extracellular proinflammatory mediators contributes to an inflammatory response in patients. Evidence exists to support an increase in in vivo proinflammatory mediators following injury as well, while suppression of genes that stimulate an anti-inflammatory response also occurs.(29) Imbalances in inflammatory cytokines can last for weeks, creating an opportunity for patients to succumb to inflammatory complications.

We found that the presence of at least one comorbid condition was independently associated with development of inflammatory complications. Smoking was the most frequently reported comorbidity. Bell, Bayt, and Zarzaur found no association between

smokers and complications after traumatic injury (smoker prevalence 9.7% versus nonsmoker prevalence 9.6%, p = 0.763).(63) Ferro and colleagues supported this finding with similar rates of sepsis, pneumonia, ARDS, and MODS among smokers and non-smokers.(64) However, numerous prior investigations have clearly implicated cigarette smoking with up-regulation of proinflammatory cytokines and down-regulation of anti-inflammatory cytokines. Prior investigators suggested that cigarette smoking was associated with prolonged bone healing after fracture, and the risk for nonunion of fracture and wound infection.(65) However, recent investigators found that after trauma cigarette smokers were 85% less likely to die and 27% less likely to develop a major complication compared with nonsmokers.(63) Thus, evidence about an association between smoking and inflammatory complications after trauma is equivocal and the proposed protective mechanism requires further investigation.(66-69)

Cigarette smoking is often associated with chronic alcoholism and psychiatric disorders,(70) two common comorbidities of our participants. Yaghoubian and colleagues (71) found that a positive blood alcohol on admission after trauma was not associated with complications. Similarly, Zeckey and colleagues (72) reported that alcohol consumption was not an independent predictor of sepsis or systemic inflammatory response syndrome in patients after trauma. However, alcohol intoxication has been demonstrated to produce dysregulation of immune mechanisms after trauma with consequent suppression of proinflammatory cytokine release, reduced neutrophil recruitment, decreased phagocytosis, impaired chemotaxis, and suppressed oxidative burst capacity;(73) thus, the likelihood of infection is augmented. Individuals with chronic and acute alcohol intoxication who have peripheral vascular injury were

demonstrated to require more surgical interventions, have more infections, vascular complications, and longer hospital stays compared with those not exposed to alcohol.(74) Ray and colleagues found that alcohol use was predictive of poorer outcomes after hip fracture; deep tissue infection and mortality rate 26 times higher than non-alcohol users were evident.(75) Crutcher and colleagues found that alcohol use increased the risk for multiple complications that included pneumonia, pulmonary embolus, and urinary tract infection in individuals after spinal cord injury.(76) Thus, alcohol, trauma, and transfusion may increase the likelihood of greater morbidity and mortality.

Injury severity was the final predictor of complications. Injury provided opportunity for complications through breaks in skin integrity, and the release of anti- and proinflammatory mediators,(29) as well as placement of invasive catheters, and exposure to multiple organisms found in the environment at the site of injury and the hospital during treatment. The NISS has been a significant predictor of both sepsis (OR 1.11, 95% CI 1.04-1.19, $p = 0.0028$) and severe systemic inflammatory response syndrome (OR 1.07, 95% CI 1.00-1.14, $p = 0.04$) in adult patients with major trauma.(77) In addition, the NISS has been associated with complicated recovery and development of MOF, where patients with uncomplicated recovery and no MOF were less injured compared to those who developed MOF (average NISS 32.6 ± 1.8 versus 39.8 ± 1.9, $p = 0.01$).(78) Therefore, our results support the construct validity of the NISS, and coincide with those in the existing trauma literature.(77, 79, 80)

Limitations

The findings of our study should be considered in light of several limitations. First, the data provided through the TRDB were limited in that the investigators selected

the variables for data collection, thereby restricting our selection for analysis. One variable of particular interest that was not available in this database was the age of the transfused components. Other variables that would have supplemented our study are timing of component transfusion in relation to time of injury and levels of inflammatory cytokines for all patients. Though a small subset of patients had data available for cytokines, these data were inadequate for our analyses.

Second, because the data were deidentified prior to release for secondary analysis, the validity could not be determined. Data entry by multiple personnel at each location lends itself to the possibility of errors. Finally, we restricted our analysis to patients between the ages of 18-65 to reduce the potential influence that older age and more comorbid conditions might introduce. However, our participants exhibited a number of comorbid conditions.

Conclusion

A majority of our participants with major trauma developed inflammatory complications during their hospitalization. The volume of PRBCs transfused in the first 24 hours of hospitalization and the presence of comorbid conditions were independently associated with the development of complications. Although the likelihood that comorbid conditions are associated with complication has been previously hypothesized, clinicians often do not have access to these data at the time of management. The severity of injury was also a predictor of inflammatory complications. Enhanced understanding of the mechanisms that contribute to immune alterations after trauma and transfusion may provide clinicians with the ability to individualize patient management and monitoring for complications to produce improved outcomes.

Table 5.1 Characteristics of the Sample (N = 1,538)

Characteristic	**Mean (SD) or n (%)**
Sex (male)	1,040 (68%)
Age (y)	39 ± 14
Ethnicity	
Caucasian	1,364 (89%)
African American	99 (7%)
Other*	67 (4%)
Number of Comorbidities	
0	598 (39%)
1	452 (29%)
2 or more	488 (32%)
Commonly Reported Comorbidities	
Smoking	457 (30%)
Chronic alcoholism	212 (14%)
Psychiatric disorder	170 (11%)
New Injury Severity Score	39 ± 13
Mechanism of Blunt Injury	
MVC - occupant	818 (53%)
MVC - motorcyclist	267 (17%)
Other**	453 (30%)
Pre-Hospital Blood (mL)	219 ± 592
Pre-Hospital Crystalloids (mL)	2,178 ± 2,236

Table 5.1, Cont.

Temperature (°F)	
Low	95.0 ± 2.3
High	100.4 ± 1.4
MAP (mmHg)	
Low	52 ± 17
High	118 ± 19
Heart Rate (bpm)	
Low	77 ± 21
High	137 ± 21
Respiratory Rate (breaths/min)	
Low	12 ± 4
High	27 ± 7
pH	
Low	7.2 ± .1
High	7.4 ± .1
Hematocrit (%)	
Low	24 ± 6
High	39 ± 5
Hemoglobin (g/dL)	11.6 ± 2.6
INR	1.4 ± .7
Base Deficit	-8.8 ± 4.4
ED GCS	8 ± 6

Table 5.1, Cont.

APACHE II GCS	5 ± 4
Transfer From Another Hospital (yes)	433 (28%)
28-Day Patient Survival	1,387 (90%)
Primary Cause of Death	
Brain death	37 (25%)
Multiple organ failure	28 (19%)
Other***	86 (57%)
Non-Infectious Complications (yes)	655 (43%)
Number of Inflammatory Complications	2 ± 2
Disposition At Initial Hospital Discharge	
Home (with or without services)	490 (32%)
Inpatient rehab facility	408 (27%)
Skilled nursing facility	362 (24%)
Other †	278 (18%)

MVC = motor vehicle collision; mL = milliliters; ºF = degrees in Fahrenheit; mmHg = millimeters of mercury; bpm = beats per minute; g/dL = grams per deciliter; mEq/L = milliequivalents per liter; ED = emergency department; APACHE = Acute Physiology and Chronic Health Enquiry II
* = American Indian, Asian, Pacific Islander, Other
** = Moving vehicle collision – cyclist, struck by or against, pedestrian; falls; machinery injuries; other
*** = Hypovolemic shock, sepsis, hypoxia, cardiac dysfunction, severe head injury (trauma only), withdrawal of life sustaining therapy, other
† = Nursing home, residential facility, against medical advice, another acute care facility, death or other

Table 5.2 Transfusion-Related Characteristics

Transfused in first 6 hours (yes)	**Mean ± SD or n (%)**
PRBC	1,411 (92%)
FFP	773 (50%)
PLT	395 (26%)
Transfusion in first 24 hours (yes)	
PRBC	1,536 (100%)
FFP	1,000 (65%)
PLT	618 (40%)
Transfusion volume in first 6 hours	
PRBC (mL)	1,977 ± 2,329
Units	6.6 ± 7.8
FFP (mL)	705 ± 1,102
Units	3.5 ± 5.5
PLT (mL)	125 ± 330
Units	2.5 ± 6.6
Transfusion volume in first 24 hours	
PRBC (mL)	2,843 ± 3,599
Units	9.5 ± 12
FFP (mL)	1,195 ± 1,899
Units	6.0 ± 9.5
PLT (mL)	242 ± 486
Units	2.5 ± 6.6

Table 5.2, Cont.

Ratios transfused components at 6 hours (units)	
PRBC:FFP	1.9 ± 1.9
PRBC:PLT	1.9 ± 2.1
Ratios transfused components at 24 hours (units)	
PRBC:FFP	1.9 ± 2.0
PRBC:PLT	2.6 ± 2.9

PRBC = packed red blood cells; PLT = platelets; FFP = fresh frozen plasma; mL = milliliters

Table 5.3 Inflammatory Complications (N = 1,538)

Complication	**N (%)**
Multiple organ failure	1,163 (76%)
Ventilator-associated pneumonia	421 (27%)
Acute respiratory distress syndrome	371 (24%)
Urinary tract infection	222 (14%)
Septicemia	197 (13%)
Catheter-related bloodstream infection	41 (3%)
Nosocomial Infection	691 (45%)

Organ failure based on Denver maximum score, categorized as those with a score of 1 or greater[81]

Table 5.4 Predictors of Development of Inflammatory Complications (n = 386)

Variable (Reference Group)	β	Exp β	95% CI	*P*
Age (years)	.031	1.032	1.000 – 1.065	**.053**
Sex (female)	.571	1.769	.821 – 3.812	.145
Comorbidities (none)	1.680	5.367	2.235 – 12.886	**< .001**
NISS ($\leq$ 24)	.846	2.329	.648 – 8.366	.195
24-hour transfused vol. PRBC units	.079	1.083	1.018 – 1.151	**.011**
24-hour transfused vol. PLT units	.035	1.035	.943 – 1.136	.464
PRBC:FFP (outside 0.5-1.5)	.057	1.059	.478 – 2.344	.888
PRBC:PLT (outside 0.5-1.5)	-.300	.741	.297 – 1.850	.521

Omnibus Tests of Model Coefficients $p < 0.001$; Hosmer and Lemeshow $p = 0.241$; NISS = New Injury Severity Score; PRBC = packed red blood cells; PLT = platelets; FFP = fresh frozen plasma. Comparison group for categorical variables provided in parentheses.

Table 5.5 Predictors of Time to Development of Inflammatory Complications (n = 643)

Variable (Reference Group)	HR	95% CI	*P*
Age (years)	.999	.992 – 1.005	.739
Sex (female)	1.197	.998 – 1.436	**.053**
Comorbidities (none)	1.163	.971 – 1.394	.101
NISS (≤ 24)	1.409	1.034 – 1.920	**.030**
24-hour transfused vol. PRBC units	1.010	1.004 – 1.016	**.001**
24-hour transfused vol. PLT units	1.003	.994 – 1.011	.533
PRBC:FFP (other than 0.5-1.5)	1.068	.900 – 1.267	.451
PRBC:PLT (other than 0.5-1.5)	.970	.809 – 1.164	.746

Omnibus Tests of Model Coefficients $p < 0.001$; NISS = New Injury Severity Score; PRBC = packed red blood cells; PLT = platelets; FFP = fresh frozen plasma. Comparison group for categorical variables provided in parentheses. Approximately 12% of patients were censored.

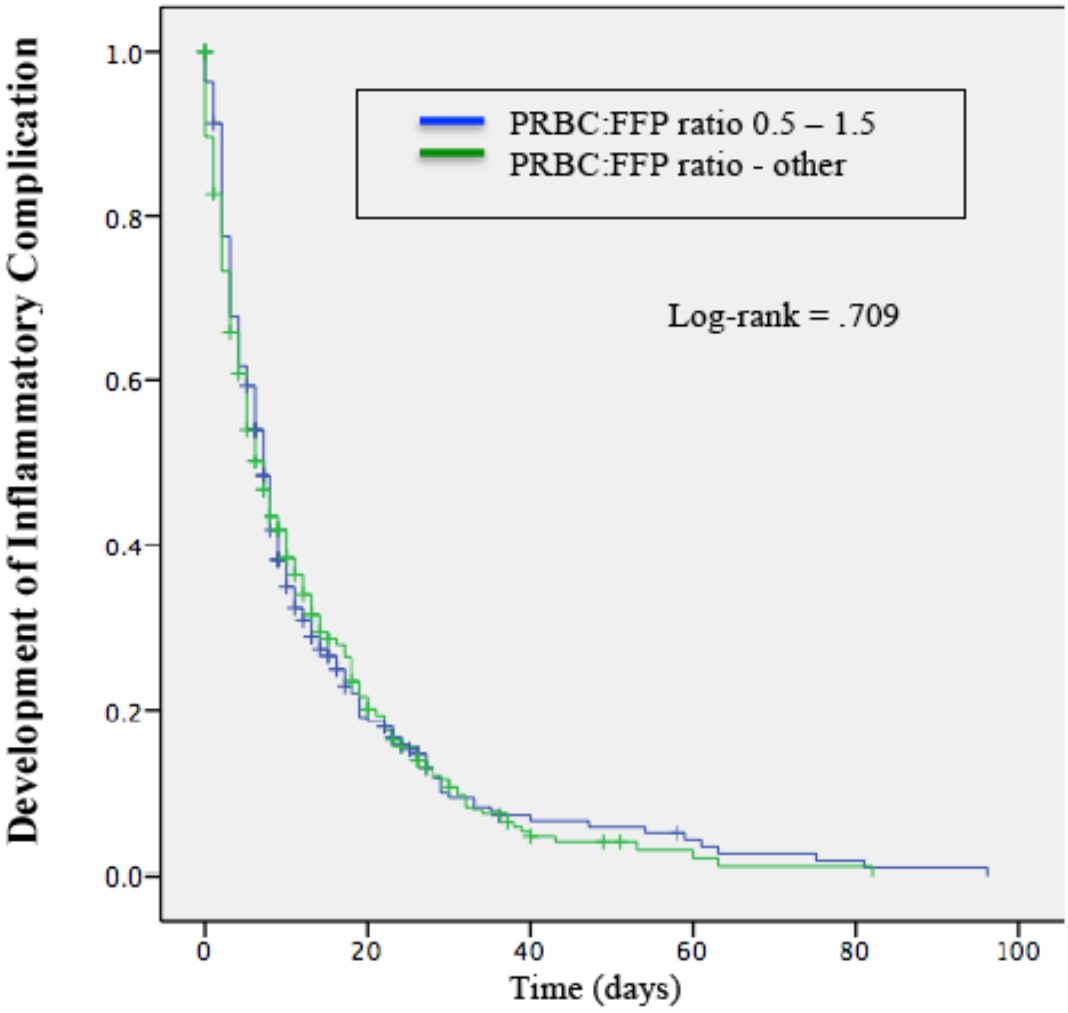


Figure 5.1 Time to development of complications by PRBC:FFP ratio group

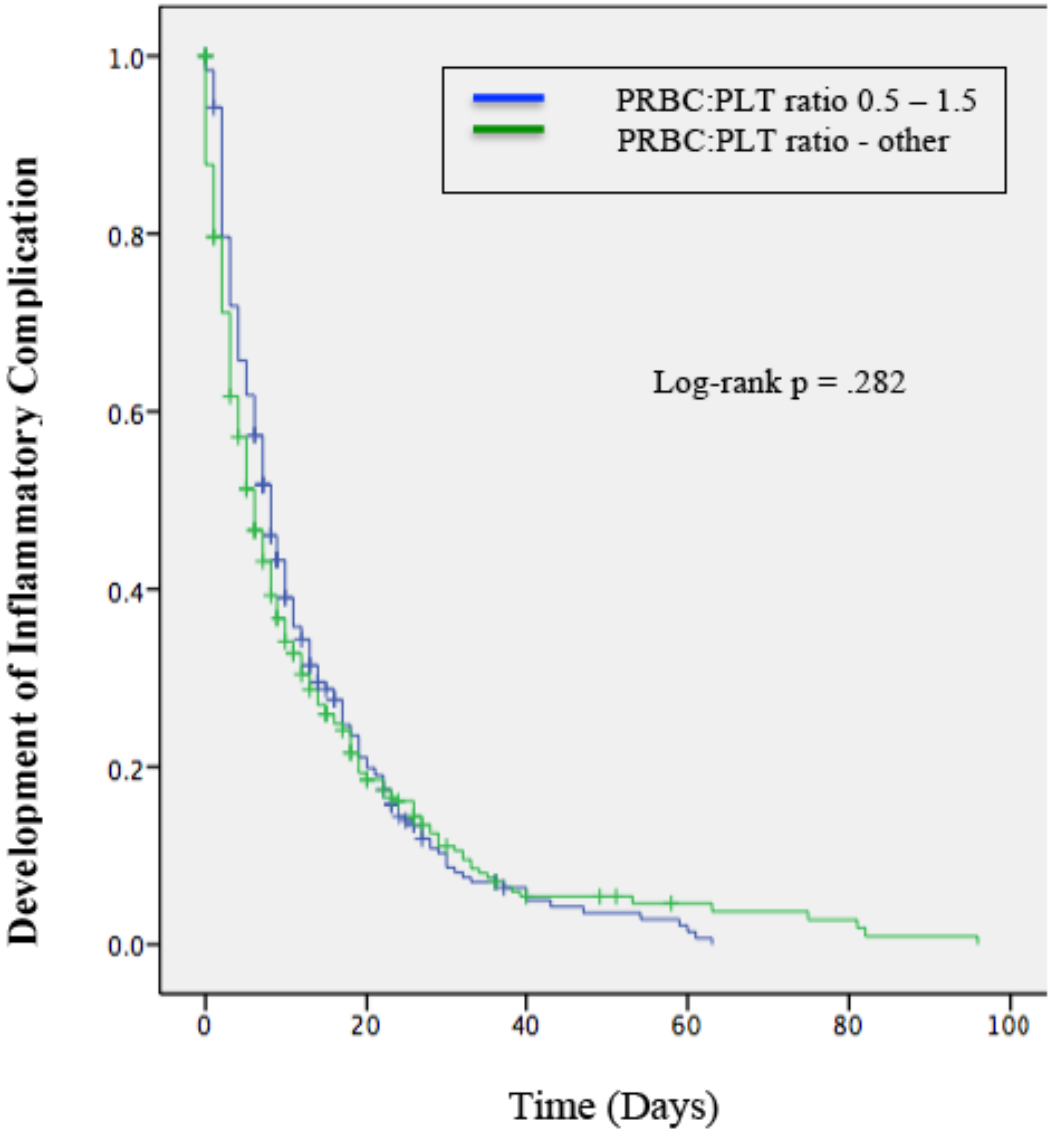


Figure 5.2 Time to development of complications by PRBC:PLT ratio group

Figure Legend

Figure 5.1 Time to development of complications by PRBC:FFP ratio group

Figure 5.2 Time to development of complications by PRBC:PLT ratio group

CHAPTER SIX

Conclusion

The purpose of this dissertation was to evaluate outcomes associated with blood component transfusions in adult major trauma patients. Unintentional traumatic injury affects millions of people worldwide annually,(1) with many patients requiring transfusion of blood or blood components for resuscitation. Stored components experience biological and chemical alterations that result in a storage lesion.(2) Evidence exists to support an increased risk of complications and mortality in transfused trauma patients,(3) yet the relationship between the storage lesion and patient outcomes remains unclear.

The first manuscript presented a conceptual model of the current state of knowledge regarding short- and long-term outcomes associated with trauma, hemorrhage, and transfusion, focusing on the consequences of the storage lesion. The second manuscript, a systematic review of the literature, focused on outcomes for massively transfused trauma patients based on the ratios of blood components they received during resuscitation. The third manuscript presented findings from a secondary analysis of the 2009 National Trauma Data Bank (NTDB) data set, evaluating mortality likelihood for trauma patients who were transfused with whole blood compared with those who received blood components. The fourth manuscript presented findings from a secondary analysis of the Inflammation and Host Response to Injury Trauma Database (TRDB), in which the relationship between blood component transfusion and development of inflammatory complications was evaluated.

The short- and long-term outcomes of traumatic injury and subsequent hemorrhage have been thoroughly discussed in the literature.(4-7) However, a lack of evidence exists regarding both short- and long-term outcomes associated with the transfusion of blood components and the storage lesion, particularly in packed red blood cells (PRBCs) and platelets (PLTs). The second manuscript presented a conceptual model depicting the state of knowledge surrounding these issues, with specific focus on the pathophysiology of the storage lesion. Consequences of the storage lesion induce a systemic inflammatory response in the transfused patient, as well as vasoconstriction,(2) electrolyte imbalances, and acidosis,(8) and these effects only worsen with time. Particular attention to patient status following trauma and subsequent transfusion is indicated in anticipation of inflammatory reactions. Suggestions for future research in this area include prevention and development of specific inflammatory complications, hospital readmission rates, rehabilitation requirements, and cognitive dysfunction.

Due to the damaging effects associated with transfusion of stored components, a systematic review of the literature was performed to evaluate outcomes of trauma patients who required massive transfusion (at least 10 units of PRBCs in a 24-hour period) based on the ratio of components transfused. This review consisted of 21 studies that were primarily retrospective in nature, and included both military and civilian populations. Risk of bias was evaluated using a 9-item instrument adapted from Viswanathan & Berkman.(9) Overall, the studies were found to have low risk of bias, but there were issues related to reporting of adverse events and outcomes, and controlling of confounding factors in the analyses. The main finding from review of the studies was that transfusion of components in a 1:1:1 ratio, or 1 unit of PRBCs to 1 unit of PLTs to 1 unit

of fresh frozen plasma (FFP), produced superior survival in massively transfused trauma patients. This ratio closely resembles the composition of whole blood, which investigators suggest may be the reason for its survival benefit. Results regarding the relationship of this ratio with complications during hospitalization were inconclusive and require further investigation.

Findings from this systematic review led to an investigation of the NTDB to evaluate mortality likelihood for major trauma patients who received whole blood compared with those who received blood components. After controlling for age, gender, Injury Severity Score, emergency medical system transfer time, and transfer of the patient from another hospital, transfusion of blood components was found to be associated with a three-fold increase in mortality likelihood in trauma patients compared with transfusion of whole blood. However, whole blood transfusion is rare in the clinical setting outside of front-line military scenarios or in surgery. Our findings call into question the current standard of care for resuscitation of patients with major trauma, including the ubiquitous transfusion of blood components. The volume and age of the transfused stored components may have influenced these outcomes, thus further investigation is warranted.

To explore further the notion that transfusion of components in a 1:1:1 fashion may be beneficial to trauma patients in terms of development of complications, the third manuscript presented findings from a secondary analysis of the TRDB. This database included only blunt trauma patients; from this sample, those who were critically injured and required blood component transfusion in the first 24 hours following hospital admission were selected. The investigation yielded several important findings. First, the vast majority of patients developed at least one inflammatory complication. Second,

though the volume of PRBCs transfused in the first 24 hours of hospitalization was associated with development of inflammatory complications, total transfused volume of FFP and PLT in the first 24 hours of hospitalization and the ratios of transfused components were not. Third, presence of at least one comorbidity increased likelihood of complication development.

Two independent predictors of time to development of inflammatory complications were identified: the initial 24-hour total transfused volume of PRBCs and injury severity. Again, the total transfused volume of FFP and PLTs in the first 24 hours of care and the ratios of PRBCs:PLTs and PRBCs:FFP were not predictors of complications. Investigators have suggested that the timing of transfusions may have more impact than the volume or ratio of components transfused in massively transfused trauma patients,(10) providing an area of future inquiry for those non-massively transfused. It may also be the case that non-massively transfused trauma patients have different transfusion requirements based on their coagulopathic state.

Thus, the findings of this dissertation support transfusion of components in a 1:1:1 ratio for survival benefit in massively transfused trauma patients, but this may not apply to trauma patients requiring less than massive transfusion. Whole blood was found to be more beneficial for survival in major trauma patients, as well. However, this is not current standard of care for resuscitation, especially in the civilian setting. In blunt trauma patients, neither the volume of transfused components nor the ratio of transfused components was associated with development of inflammatory complications, despite over 85% of transfused patients developing at least one.

Limitations of this dissertation must be considered along with the findings. The studies included in the systematic review were primarily retrospective in nature, thereby restricting investigators' abilities to control for factors that may influence patient outcomes. There was also great variability in the analyses performed by investigators and the confounding factors for which they controlled, thus limiting the generalizability of findings. The secondary analyses performed using the NTDB and the TRDB data were limited in terms of the data available for analysis. The NTDB, for example, did not include any data related to transfusion of FFP or volume of components/whole blood transfused, thereby limiting the analysis and findings. Neither database had data pertaining to age of stored components. Given that both data sets contained large numbers of transfused trauma patients, this information could have greatly supplemented both analyses. Finally, as with any retrospective analysis, missing data or incorrect data due to entry error poses a challenge, and the nature of a secondary analysis of deidentified data does not allow for determination of data validity.

The most effective transfusion strategy for survival and development of complications in both massively and non-massively transfused trauma patients has yet to be determined. Further research is required to explore the consequences of the storage lesion in transfused trauma patients. Future studies focusing on outcomes in both the short-term and long-term may help guide evaluation and treatment of the trauma population.

References

Chapter One

1. Centers for Disease Control and Prevention. 10 Leading Causes of Death by Age Group, United States - 2012. 2012; http://www.cdc.gov/injury/wisqars/pdf/leading_causes_of_death_by_age_group_2012-a.pdf. Accessed February 19, 2015.
2. Perel P, Prieto-Merino D, Shakur H, et al. Predicting early death in patients with traumatic bleeding: development and validation of prognostic model. *BMJ.* 2012;345:e5166.
3. Spinella PC. Warm fresh whole blood transfusion for severe hemorrhage: U.S. military and potential civilian applications. *Critical Care Medicine.* 2008;36(7 Suppl):S340-345.
4. Report of the US Department of Health and Human Services. *The 2011 National Blood Collection and Utilization Survey Report.* Washington, DC: U.S. Department of Health and Human Services;2013.
5. Holcomb JB. Optimal use of blood products in severely injured trauma patients. *Hematology Am Soc Hematol Educ Program.* 2010;2010:465-469.
6. Nunez TC, Dutton WD, May AK, Holcomb JB, Young PP, Cotton BA. Emergency department blood transfusion predicts early massive transfusion and early blood component requirement. *Transfusion.* 2010;50(9):1914-1920.
7. Duchesne JC, Hunt JP, Wahl G, et al. Review of current blood transfusions strategies in a mature level I trauma center: were we wrong for the last 60 years? *The Journal of Trauma.* 2008;65(2):272-276; discussion 276-278.

8. Bennett-Guerrero E, Veldman TH, Doctor A, et al. Evolution of adverse changes in stored RBCs. *Proc Natl Acad Sci U S A.* 2007;104(43):17063-17068.

9. Aubron C, Nichol A, Cooper DJ, Bellomo R. Age of red blood cells and transfusion in critically ill patients. *Annals of intensive care.* 2013;3(1):2.

10. Vraets A, Lin Y, Callum JL. Transfusion-associated hyperkalemia. *Transfus Med Rev.* 2011;25(3):184-196.

11. Patel MB, Proctor KG, Majetschak M. Extracellular ubiquitin increases in packed red blood cell units during storage. *J Surg Res.* 2006;135(2):226-232.

12. Lelubre C, Vincent JL. Relationship between red cell storage duration and outcomes in adults receiving red cell transfusions: a systematic review. *Crit Care.* 2013;17(2):R66.

13. Weinberg JA, MacLennan PA, Vandromme-Cusick MJ, et al. The deleterious effect of red blood cell storage on microvascular response to transfusion. *The journal of trauma and acute care surgery.* 2013;75(5):807-812.

14. Stan A, Zsigmond E. The restoration in vivo of 2,3-diphosphoglycerate (2,3-DPG) in stored red cells, after transfusion. The levels of red cells 2,3-DPG. *Rom J Intern Med.* 2009;47(2):173-177.

15. Seghatchian J, Putter JS. Pathogen inactivation of whole blood and red cell components: an overview of concept, design, developments, criteria of acceptability and storage lesion. *Transfus Apher Sci.* 2013;49(2):357-363.

16. Roback JD. Vascular effects of the red blood cell storage lesion. *Hematology Am Soc Hematol Educ Program.* 2011;2011:475-479.

17. Orlov D, Karkouti K. The pathophysiology and consequences of red blood cell storage. *Anaesthesia.* 2015;70 Suppl 1:29-e12.

18. Bochicchio GV, Napolitano L, Joshi M, Bochicchio K, Meyer W, Scalea TM. Outcome analysis of blood product transfusion in trauma patients: a prospective, risk-adjusted study. *World J Surg.* 2008;32(10):2185-2189.

19. Kopriva BM, Helmer SD, Smith RS. Jack A. Barney resident paper award: blood transfusions increase complications in moderately injured patients. *Am J Surg.* 2010;200(6):746-750; discussion 750-741.

20. Borgman MA, Spinella PC, Perkins JG, et al. The ratio of blood products transfused affects mortality in patients receiving massive transfusions at a combat support hospital. *The Journal of Trauma.* 2007;63(4):805-813.

21. Koch CG, Li L, Duncan AI, et al. Morbidity and mortality risk associated with red blood cell and blood-component transfusion in isolated coronary artery bypass grafting. *Crit Care Med.* 2006;34(6):1608-1616.

22. Glance LG, Dick AW, Mukamel DB, et al. Association between intraoperative blood transfusion and mortality and morbidity in patients undergoing noncardiac surgery. *Anesthesiology.* 2011;114(2):283-292.

23. Cotton BA, Podbielski J, Camp E, et al. A randomized controlled pilot trial of modified whole blood versus component therapy in severely injured patients requiring large volume transfusions. *Ann Surg.* 2013;258(4):527-532; discussion 532-523.

24. Kudo D, Sasaki J, Akaishi S, et al. Efficacy of a high FFP:PRBC transfusion ratio on the survival of severely injured patients: a retrospective study in a single tertiary emergency center in Japan. *Surg Today.* 2013.

25. Cap AP, Spinella PC, Borgman MA, Blackbourne LH, Perkins JG. Timing and location of blood product transfusion and outcomes in massively transfused combat casualties. *The journal of trauma and acute care surgery.* 2012;73(2 Suppl 1):S89-94.

26. Brown JB, Cohen MJ, Minei JP, et al. Debunking the survival bias myth: characterization of mortality during the initial 24 hours for patients requiring massive transfusion. *The journal of trauma and acute care surgery.* 2012;73(2):358-364; discussion 364.

27. Dzik WH, Blajchman MA, Fergusson D, et al. Clinical review: Canadian National Advisory Committee on Blood and Blood Products--Massive transfusion consensus conference 2011: report of the panel. *Crit Care.* 2011;15(6):242.

28. Marik PE, Corwin HL. Efficacy of red blood cell transfusion in the critically ill: a systematic review of the literature. *Critical Care Medicine.* 2008;36(9):2667-2674.

29. Perel P, Clayton T, Altman DG, et al. Red blood cell transfusion and mortality in trauma patients: risk-stratified analysis of an observational study. *PLoS Med.* 2014;11(6):e1001664.

Chapter Two

1. Centers for Disease Control and Prevention. *10 Leading Causes of Death by Age Group, United States - 2010.* National Vital Statistics System; October 15, 2012 2012.
2. Kauvar DS, Lefering R, Wade CE. Impact of hemorrhage on trauma outcome: an overview of epidemiology, clinical presentations, and therapeutic considerations. *The Journal of Trauma.* Jun 2006;60(6 Suppl):S3-11.
3. Rossaint R, Bouillon B, Cerny V, et al. Management of bleeding following major trauma: an updated European guideline. *Crit Care.* 2010;14(2):R52.
4. Brown JB, Cohen MJ, Minei JP, et al. Debunking the survival bias myth: characterization of mortality during the initial 24 hours for patients requiring massive transfusion. *The journal of trauma and acute care surgery.* Aug 2012;73(2):358-364; discussion 364.
5. Mitra B, O'Reilly G, Cameron PA, Zatta A, Gruen RL. Effectiveness of massive transfusion protocols on mortality in trauma: a systematic review and meta-analysis. *ANZ J Surg.* Dec 2013;83(12):918-923.
6. Hallet J, Lauzier F, Mailloux O, et al. The use of higher platelet: RBC transfusion ratio in the acute phase of trauma resuscitation: a systematic review. *Crit Care Med.* Dec 2013;41(12):2800-2811.
7. Bohmer AB, Just KS, Lefering R, et al. Factors influencing lengths of stay in the intensive care unit for surviving trauma patients: a retrospective analysis of 30,157 cases. *Crit Care.* Jul 7 2014;18(4):R143.

8. Watanabe S. Vocational rehabilitation for clients with cognitive and behavioral disorders associated with traumatic brain injury. *Work.* Jan 1 2013;45(2):273-277.

9. Rietdijk R, Simpson G, Togher L, Power E, Gillett L. An exploratory prospective study of the association between communication skills and employment outcomes after severe traumatic brain injury. *Brain Inj.* 2013;27(7-8):812-818.

10. O'Donnell ML, Varker T, Holmes AC, et al. Disability after injury: the cumulative burden of physical and mental health. *J Clin Psychiatry.* Feb 2013;74(2):e137-143.

11. Haagsma JA, Ringburg AN, van Lieshout EM, et al. Prevalence rate, predictors and long-term course of probable posttraumatic stress disorder after major trauma: a prospective cohort study. *BMC Psychiatry.* 2012;12:236.

12. Jackson JC, Archer KR, Bauer R, et al. A prospective investigation of long-term cognitive impairment and psychological distress in moderately versus severely injured trauma intensive care unit survivors without intracranial hemorrhage. *J Trauma.* Oct 2011;71(4):860-866.

13. Huber-Wagner S, Qvick M, Mussack T, et al. Massive blood transfusion and outcome in 1062 polytrauma patients: a prospective study based on the Trauma Registry of the German Trauma Society. *Vox sanguinis.* Jan 2007;92(1):69-78.

14. Perkins JG, Cap AP, Spinella PC, et al. An evaluation of the impact of apheresis platelets used in the setting of massively transfused trauma patients. *J Trauma.* Apr 2009;66(4 Suppl):S77-84; discussion S84-75.

15. Marik PE, Corwin HL. Efficacy of red blood cell transfusion in the critically ill: a systematic review of the literature. *Critical Care Medicine.* Sep 2008;36(9):2667-2674.

16. Gilliss BM, Looney MR, Gropper MA. Reducing noninfectious risks of blood transfusion. *Anesthesiology.* Sep 2011;115(3):635-649.

17. Toy P, Popovsky MA, Abraham E, et al. Transfusion-related acute lung injury: definition and review. *Crit Care Med.* Apr 2005;33(4):721-726.

18. Shashaty MG, Meyer NJ, Localio AR, et al. African American race, obesity, and blood product transfusion are risk factors for acute kidney injury in critically ill trauma patients. *J Crit Care.* Oct 2012;27(5):496-504.

19. Perel P, Clayton T, Altman DG, et al. Red blood cell transfusion and mortality in trauma patients: risk-stratified analysis of an observational study. *PLoS Med.* Jun 2014;11(6):e1001664.

20. Aucar JA, Sheth M. The storage lesion of packed red blood cells affects coagulation. *Surgery.* Oct 2012;152(4):697-702; discussion 702-693.

21. Bennett-Guerrero E, Veldman TH, Doctor A, et al. Evolution of adverse changes in stored RBCs. *Proc Natl Acad Sci U S A.* Oct 23 2007;104(43):17063-17068.

22. Lelubre C, Vincent JL. Relationship between red cell storage duration and outcomes in adults receiving red cell transfusions: a systematic review. *Crit Care.* 2013;17(2):R66.

23. Jackman RP, Utter GH, Muench MO, et al. Distinct roles of trauma and transfusion in induction of immune modulation after injury. *Transfusion.* Dec 2012;52(12):2533-2550.

24. Hampton DA, Lee TH, Diggs BS, McCully SP, Schreiber MA. A predictive model of early mortality in trauma patients. *Am J Surg.* May 2014;207(5):642-647; discussion 647.

25. Rowell SE, Barbosa RR, Diggs BS, et al. Effect of high product ratio massive transfusion on mortality in blunt and penetrating trauma patients. *J Trauma.* Aug 2011;71(2 Suppl 3):S353-357.

26. Malone DL, Dunne J, Tracy JK, Putnam AT, Scalea TM, Napolitano LM. Blood transfusion, independent of shock severity, is associated with worse outcome in trauma. *J Trauma.* May 2003;54(5):898-905; discussion 905-897.

27. Dutton RP, Lefering R, Lynn M. Database predictors of transfusion and mortality. *J Trauma.* Jun 2006;60(6 Suppl):S70-77.

28. Mitra B, Tullio F, Cameron PA, Fitzgerald M. Trauma patients with the 'triad of death'. *Emerg Med J.* Aug 2012;29(8):622-625.

29. National Safety Council. *Injury Facts, 2011 Edition.* Itasca, IL: National Safety Council;2011.

30. Ireland S, Endacott R, Cameron P, Fitzgerald M, Paul E. The incidence and significance of accidental hypothermia in major trauma--a prospective observational study. *Resuscitation.* Mar 2011;82(3):300-306.

31. Martin RS, Kilgo PD, Miller PR, Hoth JJ, Meredith JW, Chang MC. Injury-associated hypothermia: an analysis of the 2004 National Trauma Data Bank. *Shock.* Aug 2005;24(2):114-118.

32. Dickson SL. Understanding the oxyhemoglobin dissociation curve. *Crit Care Nurse.* Oct 1995;15(5):54-58.

33. Thorsen K, Ringdal KG, Strand K, Soreide E, Hagemo J, Soreide K. Clinical and cellular effects of hypothermia, acidosis and coagulopathy in major injury. *Br J Surg.* Jul 2011;98(7):894-907.

34. Shafi S, Elliott AC, Gentilello L. Is hypothermia simply a marker of shock and injury severity or an independent risk factor for mortality in trauma patients? Analysis of a large national trauma registry. *J Trauma.* Nov 2005;59(5):1081-1085.

35. Marietta M, Pedrazzi P, Girardis M, Busani S, Torelli G. Posttraumatic massive bleeding: a challenging multidisciplinary task. *Internal and emergency medicine.* Dec 2010;5(6):521-531.

36. Cosgriff N, Moore EE, Sauaia A, Kenny-Moynihan M, Burch JM, Galloway B. Predicting life-threatening coagulopathy in the massively transfused trauma patient: hypothermia and acidoses revisited. *J Trauma.* May 1997;42(5):857-861; discussion 861-852.

37. Engstrom M, Schott U, Romner B, Reinstrup P. Acidosis impairs the coagulation: A thromboelastographic study. *J Trauma.* Sep 2006;61(3):624-628.

38. Johansson PI, Ostrowski SR, Secher NH. Management of major blood loss: an update. *Acta Anaesthesiol Scand.* Oct 2010;54(9):1039-1049.

39. Hess JR, Brohi K, Dutton RP, et al. The coagulopathy of trauma: a review of mechanisms. *The Journal of Trauma.* Oct 2008;65(4):748-754.

40. MacLeod JB, Lynn M, McKenney MG, Cohn SM, Murtha M. Early coagulopathy predicts mortality in trauma. *J Trauma.* Jul 2003;55(1):39-44.

41. Dirkmann D, Hanke AA, Gorlinger K, Peters J. Hypothermia and acidosis synergistically impair coagulation in human whole blood. *Anesth Analg.* Jun 2008;106(6):1627-1632.

42. Hess JR, Lawson JH. The coagulopathy of trauma versus disseminated intravascular coagulation. *J Trauma.* Jun 2006;60(6 Suppl):S12-19.

43. Bolliger D, Gorlinger K, Tanaka KA. Pathophysiology and treatment of coagulopathy in massive hemorrhage and hemodilution. *Anesthesiology.* Nov 2010;113(5):1205-1219.

44. Semon G. Thromboelastography (TEG) in Trauma. In: Surgical Critical Care Evidence-Based Medicine Guidelines Committee, ed. Orlando Regional Medical Center: Department of Surgical Education; 2013.

45. Cotton BA, Faz G, Hatch QM, et al. Rapid thrombelastography delivers real-time results that predict transfusion within 1 hour of admission. *J Trauma.* Aug 2011;71(2):407-414; discussion 414-407.

46. Maani CV, DeSocio PA, Holcomb JB. Coagulopathy in trauma patients: what are the main influence factors? *Curr Opin Anaesthesiol.* Apr 2009;22(2):255-260.

47. Jackson GN, Ashpole KJ, Yentis SM. The TEG vs the ROTEM thromboelastography/thromboelastometry systems. *Anaesthesia.* Feb 2009;64(2):212-215.

48. Doran CM, Woolley T, Midwinter MJ. Feasibility of using rotational thromboelastometry to assess coagulation status of combat casualties in a deployed setting. *J Trauma.* Jul 2010;69 Suppl 1:S40-48.

49. Walsh M, Thomas SG, Howard JC, et al. Blood component therapy in trauma guided with the utilization of the perfusionist and thromboelastography. *J Extra Corpor Technol.* Sep 2011;43(3):162-167.

50. Yin J, Zhao Z, Li Y, et al. Goal-directed transfusion protocol via thrombelastography in patients with abdominal trauma: a retrospective study. *World journal of emergency surgery : WJES.* 2014;9:28.

51. Giangrande PL. The history of blood transfusion. *Br J Haematol.* Sep 2000;110(4):758-767.

52. Orlov D, Karkouti K. The pathophysiology and consequences of red blood cell storage. *Anaesthesia.* Jan 2015;70 Suppl 1:29-e12.

53. Blasi B, D'Alessandro A, Ramundo N, Zolla L. Red blood cell storage and cell morphology. *Transfus Med.* Apr 2012;22(2):90-96.

54. Karon BS, van Buskirk CM, Jaben EA, Hoyer JD, Thomas DD. Temporal sequence of major biochemical events during blood bank storage of packed red blood cells. *Blood transfusion = Trasfusione del sangue.* Oct 2012;10(4):453-461.

55. Chin-Yee IH, Gray-Statchuk L, Milkovich S, Ellis CG. Transfusion of stored red blood cells adhere in the rat microvasculature. *Transfusion.* Nov 2009;49(11):2304-2310.

56. D'Alessandro A, Liumbruno G, Grazzini G, Zolla L. Red blood cell storage: the story so far. *Blood transfusion = Trasfusione del sangue.* Apr 2010;8(2):82-88.

57. Anniss AM, Sparrow RL. Storage duration and white blood cell content of red blood cell (RBC) products increases adhesion of stored RBCs to endothelium under flow conditions. *Transfusion.* Sep 2006;46(9):1561-1567.

58. Kor DJ, Van Buskirk CM, Gajic O. Red blood cell storage lesion. *Bosn J Basic Med Sci.* Oct 2009;9 Suppl 1:21-27.

59. Almac E, Ince C. The impact of storage on red cell function in blood transfusion. *Best Pract Res Clin Anaesthesiol.* Jun 2007;21(2):195-208.

60. Stan A, Zsigmond E. The restoration in vivo of 2,3-diphosphoglycerate (2,3-DPG) in stored red cells, after transfusion. The levels of red cells 2,3-DPG. *Rom J Intern Med.* 2009;47(2):173-177.

61. Stapley R, Vittori DA, Patel RP. Biochemistry of Storage of Red Blood Cells. In: Mozzarelli A, Bettati S, eds. *Chemistry and Biochemistry of Oxygen Therapeutics: From Transfusion to Artificial Blood*. First ed. West Sussex, United Kingdom: John Wiley & Sons Ltd; 2011:232-233.

62. Yoshida T, Shevkoplyas SS. Anaerobic storage of red blood cells. *Blood transfusion = Trasfusione del sangue.* Oct 2010;8(4):220-236.

63. D'Alessandro A, Gevi F, Zolla L. Red blood cell metabolism under prolonged anaerobic storage. *Molecular bioSystems.* Jun 2013;9(6):1196-1209.

64. Dietrich HH, Ellsworth ML, Sprague RS, Dacey RG, Jr. Red blood cell regulation of microvascular tone through adenosine triphosphate. *Am J Physiol Heart Circ Physiol.* Apr 2000;278(4):H1294-1298.

65. Karger R, Lukow C, Kretschmer V. Deformability of Red Blood Cells and Correlation with ATP Content during Storage as Leukocyte-Depleted Whole Blood. *Transfusion medicine and hemotherapy : offizielles Organ der Deutschen Gesellschaft fur Transfusionsmedizin und Immunhamatologie.* Aug 2012;39(4):277-282.

66. Flatt JF, Bawazir WM, Bruce LJ. The involvement of cation leaks in the storage lesion of red blood cells. *Frontiers in physiology.* 2014;5:214.

67. Perlow M. Perfusion, hypoperfusion, and ischemia processes: the effect on bodily function. *J Infus Nurs.* Sep-Oct 2013;36(5):336-340.

68. Chaudhary R, Katharia R. Oxidative injury as contributory factor for red cells storage lesion during twenty eight days of storage. *Blood transfusion = Trasfusione del sangue.* Jan 2012;10(1):59-62.

69. Kriebardis A, Antonelou M, Stamoulis K, Papassideri I. Cell-derived microparticles in stored blood products: innocent-bystanders or effective mediators of post-transfusion reactions? *Blood transfusion = Trasfusione del sangue.* May 2012;10 Suppl 2:s25-38.

70. Singh M, Roginskaya M, Dalal S, et al. Extracellular ubiquitin inhibits beta-AR-stimulated apoptosis in cardiac myocytes: role of GSK-3beta and mitochondrial pathways. *Cardiovasc Res.* Apr 1 2010;86(1):20-28.

71. Patel MB, Proctor KG, Majetschak M. Extracellular ubiquitin increases in packed red blood cell units during storage. *J Surg Res.* Oct 2006;135(2):226-232.

72. Devine DV, Serrano K. The platelet storage lesion. *Clinics in laboratory medicine.* Jun 2010;30(2):475-487.

73. Shrivastava M. The platelet storage lesion. *Transfus Apher Sci.* Oct 2009;41(2):105-113.

74. Jackson SP, Schoenwaelder SM. Procoagulant platelets: are they necrotic? *Blood.* 2010;116(12):2011-2018.

75. American Association of Blood Banks. Transfusion Medicine: Blood FAQ. 2014; http://www.aabb.org/tm/Pages/bloodfaq.aspx#a10. Accessed September 17, 2014.

76. Pidcoke HF, Spinella PC, Ramasubramanian AK, et al. Refrigerated platelets for the treatment of acute bleeding: a review of the literature and reexamination of current standards. *Shock.* May 2014;41 Suppl 1:51-53.

77. Rumjantseva V, Grewal PK, Wandall HH, et al. Dual roles for hepatic lectin receptors in the clearance of chilled platelets. *Nat Med.* Nov 2009;15(11):1273-1280.

78. Saunders C, Rowe G, Wilkins K, Collins P. Impact of glucose and acetate on the characteristics of the platelet storage lesion in platelets suspended in additive solutions with minimal plasma. *Vox Sang.* Jul 2013;105(1):1-10.

79. Morrell CN. Immunomodulatory mediators in platelet transfusion reactions. *Hematology Am Soc Hematol Educ Program.* 2011;2011:470-474.

80. Inaba K, Branco BC, Rhee P, et al. Impact of the duration of platelet storage in critically ill trauma patients. *J Trauma.* Dec 2011;71(6):1766-1773; discussion 1773-1764.

81. Sanderson TH, Reynolds CA, Kumar R, Przyklenk K, Huttemann M. Molecular mechanisms of ischemia-reperfusion injury in brain: pivotal role of the mitochondrial membrane potential in reactive oxygen species generation. *Mol Neurobiol.* Feb 2013;47(1):9-23.

82. Ho JJ, Man HS, Marsden PA. Nitric oxide signaling in hypoxia. *J Mol Med (Berl).* Mar 2012;90(3):217-231.

83. Valparaiso AP, Vicente DA, Bograd BA, Elster EA, Davis TA. Modeling acute traumatic injury. *J Surg Res.* Oct 22 2014.

84. Kunimatsu T, Kobayashi K, Yamashita A, Yamamoto T, Lee MC. Cerebral reactive oxygen species assessed by electron spin resonance spectroscopy in the initial stage of ischemia-reperfusion are not associated with hypothermic neuroprotection. *Journal of clinical neuroscience : official journal of the Neurosurgical Society of Australasia.* Apr 2011;18(4):545-548.

85. Nguyen KA, Hamzeh-Cognasse H, Sebban M, et al. A computerized prediction model of hazardous inflammatory platelet transfusion outcomes. *PLoS One.* 2014;9(5):e97082.

86. Burnouf T, Goubran HA, Chou ML, Devos D, Radosevic M. Platelet microparticles: detection and assessment of their paradoxical functional roles in disease and regenerative medicine. *Blood Rev.* Jul 2014;28(4):155-166.

87. Burger D, Schock S, Thompson CS, Montezano AC, Hakim AM, Touyz RM. Microparticles: biomarkers and beyond. *Clin Sci (Lond).* Apr 2013;124(7):423-441.

88. Vamvakas EC, Blajchman MA. Transfusion-related immunomodulation (TRIM): an update. *Blood Rev.* Nov 2007;21(6):327-348.

89. Xiao W, Mindrinos MN, Seok J, et al. A genomic storm in critically injured humans. *J Exp Med.* Dec 19 2011;208(13):2581-2590.

90. Wang D, Sun J, Solomon SB, Klein HG, Natanson C. Transfusion of older stored blood and risk of death: a meta-analysis. *Transfusion.* Jun 2012;52(6):1184-1195.

91. American Association of Blood Banks. Circular of Information for the Use of Human Blood and Blood Components. In: American Association of Blood Banks, ed2013.

Chapter Three

1. Murphy SL, Xu JQ, Kochanek KD. *Deaths: Final Data for 2010.* Hyattsville, MD: National Center for Health Statistics; May 8, 2013. 2013.
2. NCHS Vital Statistics System. *2011, United States: All Injury Deaths and Rates per 100,000.* National Center for Injury Prevention and Control: CDC; 2011.
3. Cripps MW, Kutcher ME, Daley A, et al. Cause and timing of death in massively transfused trauma patients. *J Trauma Acute Care Surg.* 2013;75(2 Suppl 2):S255-262.
4. Johansson PI, Stissing T, Bochsen L, Ostrowski SR. Thrombelastography and tromboelastometry in assessing coagulopathy in trauma. *Scand J Trauma Resusc Emerg Med.* 2009;17:45.
5. Geeraedts LM, Jr., Kaasjager HA, van Vugt AB, Frolke JP. Exsanguination in trauma: A review of diagnostics and treatment options. *Injury.* 2009;40(1):11-20.
6. Cohen MJ, Kutcher M, Redick B, et al. Clinical and mechanistic drivers of acute traumatic coagulopathy. *J Trauma Acute Care Surg.* 2013;75(1 Suppl 1):S40-47.
7. Brohi K, Singh J, Heron M, Coats T. Acute traumatic coagulopathy. *J Trauma.* 2003;54(6):1127-1130.
8. Perel P, Clayton T, Altman DG, et al. Red blood cell transfusion and mortality in trauma patients: risk-stratified analysis of an observational study. *PLoS Med.* 2014;11(6):e1001664.
9. Report of the US Department of Health and Human Services. *The 2011 National Blood Collection and Utilization Survey Report.* Washington, DC: U.S. Department of Health and Human Services;2013.

10. Cotton BA, Dossett LA, Haut ER, et al. Multicenter validation of a simplified score to predict massive transfusion in trauma. *J Trauma.* 2010;69 Suppl 1:S33-39.

11. Sadjadi J, Cureton EL, Twomey P, Victorino GP. Transfusion, not just injury severity, leads to posttrauma infection: a matched cohort study. *The American Surgeon.* 2009;75(4):307-312.

12. DeLoughery TG. Logistics of massive transfusions. *Hematology Am Soc Hematol Educ Program.* 2010;2010:470-473.

13. Schuster KM, Davis KA, Lui FY, Maerz LL, Kaplan LJ. The status of massive transfusion protocols in United States trauma centers: massive transfusion or massive confusion? *Transfusion.* 2010;50(7):1545-1551.

14. Viswanathan M, Berkman ND. Development of the RTI item bank on risk of bias and precision of observational studies. *J Clin Epidemiol.* 2012;65(2):163-178.

15. Inaba K, Lustenberger T, Rhee P, et al. The impact of platelet transfusion in massively transfused trauma patients. *J Am Coll Surg.* 2010;211(5):573-579.

16. Sperry JL, Ochoa JB, Gunn SR, et al. An FFP:PRBC transfusion ratio >/=1:1.5 is associated with a lower risk of mortality after massive transfusion. *J Trauma.* 2008;65(5):986-993.

17. Borgman MA, Spinella PC, Perkins JG, et al. The ratio of blood products transfused affects mortality in patients receiving massive transfusions at a combat support hospital. *J Trauma.* 2007;63(4):805-813.

18. Duchesne JC, Hunt JP, Wahl G, et al. Review of current blood transfusions strategies in a mature level I trauma center: were we wrong for the last 60 years? *J Trauma.* 2008;65(2):272-276; discussion 276-278.

19. Gunter OL, Jr., Au BK, Isbell JM, Mowery NT, Young PP, Cotton BA. Optimizing outcomes in damage control resuscitation: identifying blood product ratios associated with improved survival. *J Trauma.* 2008;65(3):527-534.

20. Holcomb JB, Wade CE, Michalek JE, et al. Increased plasma and platelet to red blood cell ratios improves outcome in 466 massively transfused civilian trauma patients. *Ann Surg.* 2008;248(3):447-458.

21. Kashuk JL, Moore EE, Johnson JL, et al. Postinjury life threatening coagulopathy: is 1:1 fresh frozen plasma:packed red blood cells the answer? *J Trauma.* 2008;65(2):261-270; discussion 270-261.

22. Maegele M, Lefering R, Paffrath T, Tjardes T, Simanski C, Bouillon B. Red-blood-cell to plasma ratios transfused during massive transfusion are associated with mortality in severe multiple injury: a retrospective analysis from the Trauma Registry of the Deutsche Gesellschaft fur Unfallchirurgie. *Vox Sang.* 2008;95(2):112-119.

23. Duchesne JC, Islam TM, Stuke L, et al. Hemostatic resuscitation during surgery improves survival in patients with traumatic-induced coagulopathy. *J Trauma.* 2009;67(1):33-37; discussion 37-39.

24. Perkins JG, Cap AP, Spinella PC, et al. An evaluation of the impact of apheresis platelets used in the setting of massively transfused trauma patients. *J Trauma.* 2009;66(4 Suppl):S77-84; discussion S84-75.

25. Snyder CW, Weinberg JA, McGwin G, Jr., et al. The relationship of blood product ratio to mortality: survival benefit or survival bias? *J Trauma.* 2009;66(2):358-362; discussion 362-354.

26. Teixeira PG, Inaba K, Shulman I, et al. Impact of plasma transfusion in massively transfused trauma patients. *J Trauma.* 2009;66(3):693-697.

27. Zink KA, Sambasivan CN, Holcomb JB, Chisholm G, Schreiber MA. A high ratio of plasma and platelets to packed red blood cells in the first 6 hours of massive transfusion improves outcomes in a large multicenter study. *Am J Surg.* 2009;197(5):565-570; discussion 570.

28. Mitra B, Mori A, Cameron PA, Fitzgerald M, Paul E, Street A. Fresh frozen plasma (FFP) use during massive blood transfusion in trauma resuscitation. *Injury.* 2010;41(1):35-39.

29. Van PY, Sambasivan CN, Wade CE, et al. High transfusion ratios are not associated with increased complication rates in patients with severe extremity injuries. *J Trauma.* 2010;69 Suppl 1:S64-68.

30. Holcomb JB, Zarzabal LA, Michalek JE, et al. Increased platelet:RBC ratios are associated with improved survival after massive transfusion. *J Trauma.* 2011;71(2 Suppl 3):S318-328.

31. Magnotti LJ, Zarzaur BL, Fischer PE, et al. Improved survival after hemostatic resuscitation: does the emperor have no clothes? *J Trauma.* 2011;70(1):97-102.

32. Peiniger S, Nienaber U, Lefering R, et al. Balanced massive transfusion ratios in multiple injury patients with traumatic brain injury. *Crit Care.* 2011;15(1):R68.

33. Brown JB, Cohen MJ, Minei JP, et al. Debunking the survival bias myth: characterization of mortality during the initial 24 hours for patients requiring massive transfusion. *J Trauma Acute Care Surg.* 2012;73(2):358-364; discussion 364.

34. Cap AP, Spinella PC, Borgman MA, Blackbourne LH, Perkins JG. Timing and location of blood product transfusion and outcomes in massively transfused combat casualties. *J Trauma Acute Care Surg.* 2012;73(2 Suppl 1):S89-94.

35. Kudo D, Sasaki J, Akaishi S, et al. Efficacy of a high FFP:PRBC transfusion ratio on the survival of severely injured patients: a retrospective study in a single tertiary emergency center in Japan. *Surg Today.* 2013.

36. Hunt JP, Weintraub SL, Wang YZ, Buechter KJ. Kinematics of trauma. *Trauma*. 5th ed. New York, NY: McGraw-Hill Companies, Inc.; 2004.

37. Rowell SE, Barbosa RR, Diggs BS, et al. Effect of high product ratio massive transfusion on mortality in blunt and penetrating trauma patients. *J Trauma.* 2011;71(2 Suppl 3):S353-357.

38. Jones AR, Frazier SK. Increased mortality in adult patients with trauma transfused with blood components compared with whole blood. *J Trauma Nurs.* 2014;21(1):22-29.

39. Spinella PC, Perkins JG, Grathwohl KW, Beekley AC, Holcomb JB. Warm fresh whole blood is independently associated with improved survival for patients with combat-related traumatic injuries. *J Trauma.* 2009;66(4 Suppl):S69-76.

40. Seghatchian J, Samama MM. Massive transfusion: an overview of the main characteristics and potential risks associated with substances used for correction of a coagulopathy. *Transfus Apher Sci.* 2012;47(2):235-243.

41. Dente CJ, Shaz BH, Nicholas JM, et al. Improvements in early mortality and coagulopathy are sustained better in patients with blunt trauma after institution of a massive transfusion protocol in a civilian level I trauma center. *J Trauma.* 2009;66(6):1616-1624.

42. Cotton BA, Au BK, Nunez TC, Gunter OL, Robertson AM, Young PP. Predefined massive transfusion protocols are associated with a reduction in organ failure and postinjury complications. *J Trauma.* 2009;66(1):41-48; discussion 48-49.

43. American College of Surgeons. Product Catalog. 2013; https://web4.facs.org/ebusiness/ProductCatalog/ProductCategory.aspx?id=26. Accessed December 4, 2013.

44. Sorensen B, Fries D. Emerging treatment strategies for trauma-induced coagulopathy. *Br J Surg.* 2012;99 Suppl 1:40-50.

45. Neal MD, Marsh A, Marino R, et al. Massive transfusion: an evidence-based review of recent developments. *Arch Surg.* 2012;147(6):563-571.

46. del Junco DJ, Holcomb JB, Fox EE, et al. Resuscitate early with plasma and platelets or balance blood products gradually: findings from the PROMMTT study. *J Trauma Acute Care Surg.* 2013;75(1 Suppl 1):S24-30.

47. Rowell SE, Barbosa RR, Allison CE, et al. Gender-based differences in mortality in response to high product ratio massive transfusion. *J Trauma.* 2011;71(2 Suppl 3):S375-379.

48. Riskin DJ, Tsai TC, Riskin L, et al. Massive transfusion protocols: the role of aggressive resuscitation versus product ratio in mortality reduction. *J Am Coll Surg.* 2009;209(2):198-205.

49. Ho AM, Dion PW, Yeung JH, et al. Prevalence of survivor bias in observational studies on fresh frozen plasma:erythrocyte ratios in trauma requiring massive transfusion. *Anesthesiology.* 2012;116(3):716-728.

50. del Junco DJ, Fox EE, Camp EA, Rahbar MH, Holcomb JB. Seven deadly sins in trauma outcomes research: an epidemiologic post mortem for major causes of bias. *J Trauma Acute Care Surg.* 2013;75(1 Suppl 1):S97-103.

51. Dzik WH, Blajchman MA, Fergusson D, et al. Clinical review: Canadian National Advisory Committee on Blood and Blood Products--Massive transfusion consensus conference 2011: report of the panel. *Crit Care.* 2011;15(6):242.

52. Nascimento B, Rizoli S, Rubenfeld G, Lin Y, Callum J, Tien HC. Design and preliminary results of a pilot randomized controlled trial on a 1:1:1 transfusion strategy: the trauma formula-driven versus laboratory-guided study. *J Trauma.* 2011;71(5 Suppl 1):S418-426.

Chapter Four

1. Centers for Disease Control and Prevention. *National Vital Statistics Reports*: United States Department of Health and Human Services: National Center for Heatlh Statistics, National Vital Statistics System;2011.
2. Holcomb JB, Spinella PC. Optimal use of blood in trauma patients. *Biologicals.* Jan 2010;38(1):72-77.
3. Teixeira PG, Inaba K, Hadjizacharia P, et al. Preventable or potentially preventable mortality at a mature trauma center. *J Trauma.* Dec 2007;63(6):1338-1346; discussion 1346-1337.
4. Gruen RL, Jurkovich GJ, McIntyre LK, Foy HM, Maier RV. Patterns of errors contributing to trauma mortality: lessons learned from 2,594 deaths. *Ann Surg.* Sep 2006;244(3):371-380.
5. Kauvar DS, Holcomb JB, Norris GC, Hess JR. Fresh whole blood transfusion: a controversial military practice. *J Trauma.* Jul 2006;61(1):181-184.
6. American Red Cross. Blood Facts and Statistics. 2006; http://www.redcrossblood.org/learn-about-blood/blood-facts-and-statistics - blood-components. Accessed September 3, 2012.
7. United States Department of Health and Human Services. *The 2009 National Blood Collection and Utilization Survey Report*2009.
8. Whitaker BI, Hinkins S. *The 2011 National Blood Collection and Utilization Survey Report*: U.S. Department of Health and Human Services;2011.

9. Glance LG, Dick AW, Mukamel DB, et al. Association between intraoperative blood transfusion and mortality and morbidity in patients undergoing noncardiac surgery. *Anesthesiology.* Feb 2011;114(2):283-292.

10. Vamvakas EC, Blajchman MA. Transfusion-related mortality: the ongoing risks of allogeneic blood transfusion and the available strategies for their prevention. *Blood.* Apr 9 2009;113(15):3406-3417.

11. Kapan M, Onder A, Oguz A, et al. The effective risk factors on mortality in patients undergoing damage control surgery. *Eur Rev Med Pharmacol Sci.* Jun 2013;17(12):1681-1687.

12. Cripps MW, Kutcher ME, Daley A, et al. Cause and timing of death in massively transfused trauma patients. *The journal of trauma and acute care surgery.* Aug 2013;75(2 Suppl 2):S255-262.

13. Kutcher ME, Kornblith LZ, Narayan R, et al. A Paradigm Shift in Trauma Resuscitation: Evaluation of Evolving Massive Transfusion Practices. *JAMA surgery.* Jul 17 2013.

14. Holcomb JB. Optimal use of blood products in severely injured trauma patients. *Hematology Am Soc Hematol Educ Program.* 2010;2010:465-469.

15. Spinella PC, Perkins JG, Grathwohl KW, Beekley AC, Holcomb JB. Warm fresh whole blood is independently associated with improved survival for patients with combat-related traumatic injuries. *J Trauma.* Apr 2009;66(4 Suppl):S69-76.

16. Nessen SC, Eastridge BJ, Cronk D, et al. Fresh whole blood use by forward surgical teams in Afghanistan is associated with improved survival compared to

component therapy without platelets. *Transfusion.* Jan 2013;53 Suppl 1:107S-113S.

17. Committee on Trauma ACoS. NTDB Admission Year 2008. 9.0 ed. Chicago, IL: The content reproduced from the NTDB remains the full and exclusive copyrighted property of the American College of Surgeons. The American College of Surgeons is not responsible for any claims arising from works based on the original data, text, tables, or figures.; 2010.
18. Nathens A, Fantus RJ, eds. *National Trauma Data Bank Annual Report 2009*: American College of Surgeons; 2009.
19. Kuhne CA, Ruchholtz S, Kaiser GM, Nast-Kolb D. Mortality in severely injured elderly trauma patients--when does age become a risk factor? *World J Surg.* Nov 2005;29(11):1476-1482.
20. Mostafa G, Huynh T, Sing RF, Miles WS, Norton HJ, Thomason MH. Gender-related outcomes in trauma. *J Trauma.* Sep 2002;53(3):430-434; discussion 434-435.
21. Gannon CJ, Pasquale M, Tracy JK, McCarter RJ, Napolitano LM. Male gender is associated with increased risk for postinjury pneumonia. *Shock.* May 2004;21(5):410-414.
22. Magnotti LJ, Fischer PE, Zarzaur BL, Fabian TC, Croce MA. Impact of gender on outcomes after blunt injury: a definitive analysis of more than 36,000 trauma patients. *J Am Coll Surg.* May 2008;206(5):984-991; discussion 991-982.

23. Berry C, Ley EJ, Tillou A, Cryer G, Margulies DR, Salim A. The effect of gender on patients with moderate to severe head injuries. *J Trauma.* Nov 2009;67(5):950-953.

24. Haider AH, Crompton JG, Chang DC, et al. Evidence of hormonal basis for improved survival among females with trauma-associated shock: an analysis of the National Trauma Data Bank. *J Trauma.* Sep 2010;69(3):537-540.

25. Baker SP, O'Neill B, Haddon W, Jr., Long WB. The injury severity score: a method for describing patients with multiple injuries and evaluating emergency care. *J Trauma.* Mar 1974;14(3):187-196.

26. Galvagno SM, Jr., Thomas S, Stephens C, et al. Helicopter emergency medical services for adults with major trauma. *Cochrane Database Syst Rev.* 2013;3:CD009228.

27. Wong TH, Lumsdaine W, Hardy BM, Lee K, Balogh ZJ. The impact of specialist trauma service on major trauma mortality. *The journal of trauma and acute care surgery.* Mar 2013;74(3):780-784.

28. Dewar DC, Tarrant SM, King KL, Balogh ZJ. Changes in the epidemiology and prediction of multiple-organ failure after injury. *The journal of trauma and acute care surgery.* Mar 2013;74(3):774-779.

29. Stahel PF, VanderHeiden T, Flierl MA, et al. The impact of a standardized "spine damage-control" protocol for unstable thoracic and lumbar spine fractures in severely injured patients: a prospective cohort study. *The journal of trauma and acute care surgery.* Feb 2013;74(2):590-596.

30. Curtis KA. Injury trends and mortality in adult patients with major trauma in New South Wales. *Med J Aust.* May 20 2013;198(9):481.

31. American College of Surgeons. *National Trauma Data Bank: NTDB Research Data Set Admission Year 2008 User Manual.* Chicago, IL2008.

32. Chawda MN, Hildebrand F, Pape HC, Giannoudis PV. Predicting outcome after multiple trauma: which scoring system? *Injury.* Apr 2004;35(4):347-358.

33. Semmlow JL, Cone R. Utility of the injury severity score: a confirmation. *Health Serv Res.* Spring 1976;11(1):45-52.

34. Cayten CG, Evans W. Severity indices and their implications for emergency medical services research and evaluation. *J Trauma.* Feb 1979;19(2):98-102.

35. Kim YJ. Injury severity scoring systems: a review of application to practice. *Nurs Crit Care.* May-Jun 2012;17(3):138-150.

36. Gonzalez RP, Cummings GR, Phelan HA, Mulekar MS, Rodning CB. Does increased emergency medical services prehospital time affect patient mortality in rural motor vehicle crashes? A statewide analysis. *Am J Surg.* Jan 2009;197(1):30-34.

37. Haas B, Gomez D, Zagorski B, Stukel TA, Rubenfeld GD, Nathens AB. Survival of the fittest: the hidden cost of undertriage of major trauma. *J Am Coll Surg.* Dec 2010;211(6):804-811.

38. Seghatchian J, Samama MM. Massive transfusion: an overview of the main characteristics and potential risks associated with substances used for correction of a coagulopathy. *Transfus Apher Sci.* Oct 2012;47(2):235-243.

39. Makley AT, Goodman MD, Friend LA, et al. Resuscitation with fresh whole blood ameliorates the inflammatory response after hemorrhagic shock. *J Trauma.* Feb 2010;68(2):305-311.

40. Ho KM, Leonard AD. Lack of effect of unrefrigerated young whole blood transfusion on patient outcomes after massive transfusion in a civilian setting. *Transfusion.* Aug 2011;51(8):1669-1675.

41. Aucar JA, Sheth M. The storage lesion of packed red blood cells affects coagulation. *Surgery.* Oct 2012;152(4):697-702; discussion 702-693.

42. Chin-Yee IH, Gray-Statchuk L, Milkovich S, Ellis CG. Transfusion of stored red blood cells adhere in the rat microvasculature. *Transfusion.* Nov 2009;49(11):2304-2310.

43. D'Alessandro A, Liumbruno G, Grazzini G, Zolla L. Red blood cell storage: the story so far. *Blood transfusion = Trasfusione del sangue.* Apr 2010;8(2):82-88.

44. Jy W, Ricci M, Shariatmadar S, Gomez-Marin O, Horstman LH, Ahn YS. Microparticles in stored red blood cells as potential mediators of transfusion complications. *Transfusion.* Apr 2011;51(4):886-893.

45. Bennett-Guerrero E, Veldman TH, Doctor A, et al. Evolution of adverse changes in stored RBCs. *Proc Natl Acad Sci U S A.* Oct 23 2007;104(43):17063-17068.

46. Aubron C, Nichol A, Cooper DJ, Bellomo R. Age of red blood cells and transfusion in critically ill patients. *Annals of intensive care.* 2013;3(1):2.

47. Vraets A, Lin Y, Callum JL. Transfusion-associated hyperkalemia. *Transfus Med Rev.* Jul 2011;25(3):184-196.

48. Patel MB, Proctor KG, Majetschak M. Extracellular ubiquitin increases in packed red blood cell units during storage. *J Surg Res.* Oct 2006;135(2):226-232.

49. Luten M, Roerdinkholder-Stoelwinder B, Schaap NP, de Grip WJ, Bos HJ, Bosman GJ. Survival of red blood cells after transfusion: a comparison between red cells concentrates of different storage periods. *Transfusion.* Jul 2008;48(7):1478-1485.

50. Inaba K, Lustenberger T, Rhee P, et al. The impact of platelet transfusion in massively transfused trauma patients. *Journal of the American College of Surgeons.* Nov 2010;211(5):573-579.

51. Teixeira PG, Inaba K, Shulman I, et al. Impact of plasma transfusion in massively transfused trauma patients. *J Trauma.* Mar 2009;66(3):693-697.

52. Perkins JG, Cap AP, Spinella PC, et al. An evaluation of the impact of apheresis platelets used in the setting of massively transfused trauma patients. *The Journal of Trauma.* Apr 2009;66(4 Suppl):S77-84; discussion S84-75.

53. Dirks J, Jorgensen H, Jensen CH, Ostrowski SR, Johansson PI. Blood product ratio in acute traumatic coagulopathy--effect on mortality in a Scandinavian level 1 trauma centre. *Scandinavian journal of trauma, resuscitation and emergency medicine.* 2010;18:65.

54. Bradley M, Okoye O, Dubose J, et al. Risk factors for post-traumatic pneumonia in patients with retained haemothorax: Results of a prospective, observational AAST study. *Injury.* Sep 2013;44(9):1159-1164.

55. Matityahu A, Elson J, Morshed S, Marmor M. Survivorship and severe complications are worse for octogenarians and elderly patients with pelvis

fractures as compared to adults: data from the national trauma data bank. *Journal of osteoporosis.* 2012;2012:475739.

56. Kisat M, Villegas CV, Onguti S, et al. Predictors of sepsis in moderately severely injured patients: an analysis of the National Trauma Data Bank. *Surg Infect (Larchmt).* Feb 2013;14(1):62-68.

57. Dutton RP, Lefering R, Lynn M. Database predictors of transfusion and mortality. *J Trauma.* Jun 2006;60(6 Suppl):S70-77.

58. Eastridge BJ, Malone D, Holcomb JB. Early predictors of transfusion and mortality after injury: a review of the data-based literature. *J Trauma.* Jun 2006;60(6 Suppl):S20-25.

59. Hampton DA, Fabricant LJ, Differding J, et al. Prehospital intravenous fluid is associated with increased survival in trauma patients. *The journal of trauma and acute care surgery.* Jul 2013;75(1 Suppl 1):S9-15.

60. Bernard SA, Nguyen V, Cameron P, et al. Prehospital rapid sequence intubation improves functional outcome for patients with severe traumatic brain injury: a randomized controlled trial. *Ann Surg.* Dec 2010;252(6):959-965.

61. Johnson NJ, Carr BG, Salhi R, Holena DN, Wolff C, Band RA. Characteristics and outcomes of injured patients presenting by private vehicle in a state trauma system. *Am J Emerg Med.* Feb 2013;31(2):275-281.

62. McCoy CE, Menchine M, Sampson S, Anderson C, Kahn C. Emergency medical services out-of-hospital scene and transport times and their association with mortality in trauma patients presenting to an urban Level I trauma center. *Ann Emerg Med.* Feb 2013;61(2):167-174.

63. Newgard CD, Schmicker RH, Hedges JR, et al. Emergency medical services intervals and survival in trauma: assessment of the "golden hour" in a North American prospective cohort. *Ann Emerg Med.* Mar 2010;55(3):235-246 e234.

64. Brasel KJ, Guse C, Gentilello LM, Nirula R. Heart rate: is it truly a vital sign? *J Trauma.* Apr 2007;62(4):812-817.

65. Convertino VA, Ryan KL, Rickards CA, et al. Physiological and medical monitoring for en route care of combat casualties. *J Trauma.* Apr 2008;64(4 Suppl):S342-353.

Chapter Five

1. World Health Organization. Health Topics: Injuries. 2015; http://www.who.int/topics/injuries/en/. Accessed February 22, 2015.
2. Kauvar DS, Holcomb JB, Norris GC, Hess JR. Fresh whole blood transfusion: a controversial military practice. *J Trauma.* 2006;61(1):181-184.
3. Garrioch MA. The body's response to blood loss. *Vox Sang.* 2004;87 Suppl1:74-76.
4. Tachon G, Harrois A, Tanaka S, et al. Microcirculatory alterations in traumatic hemorrhagic shock. *Crit Care Med.* 2014;42(6):1433-1441.
5. Kapan M, Onder A, Oguz A, et al. The effective risk factors on mortality in patients undergoing damage control surgery. *Eur Rev Med Pharmacol Sci.* 2013;17(12):1681-1687.
6. Cripps MW, Kutcher ME, Daley A, et al. Cause and timing of death in massively transfused trauma patients. *The journal of trauma and acute care surgery.* 2013;75(2 Suppl 2):S255-262.
7. Jones AR, Frazier SK. Increased mortality in adult patients with trauma transfused with blood components compared with whole blood. *J Trauma Nurs.* 2014;21(1):22-29.
8. Koch CG, Li L, Duncan AI, et al. Morbidity and mortality risk associated with red blood cell and blood-component transfusion in isolated coronary artery bypass grafting. *Crit Care Med.* 2006;34(6):1608-1616.

9. Kopriva BM, Helmer SD, Smith RS. Jack A. Barney resident paper award: blood transfusions increase complications in moderately injured patients. *Am J Surg.* 2010;200(6):746-750; discussion 750-741.

10. Bochicchio GV, Napolitano L, Joshi M, Bochicchio K, Meyer W, Scalea TM. Outcome analysis of blood product transfusion in trauma patients: a prospective, risk-adjusted study. *World J Surg.* 2008;32(10):2185-2189.

11. Holcomb JB, Jenkins D, Rhee P, et al. Damage control resuscitation: directly addressing the early coagulopathy of trauma. *The Journal of Trauma.* 2007;62(2):307-310.

12. Borgman MA, Spinella PC, Perkins JG, et al. The ratio of blood products transfused affects mortality in patients receiving massive transfusions at a combat support hospital. *The Journal of Trauma.* 2007;63(4):805-813.

13. Duchesne JC, Hunt JP, Wahl G, et al. Review of current blood transfusions strategies in a mature level I trauma center: were we wrong for the last 60 years? *The Journal of Trauma.* 2008;65(2):272-276; discussion 276-278.

14. Gunter OL, Jr., Au BK, Isbell JM, Mowery NT, Young PP, Cotton BA. Optimizing outcomes in damage control resuscitation: identifying blood product ratios associated with improved survival. *The Journal of Trauma.* 2008;65(3):527-534.

15. Maegele M, Lefering R, Paffrath T, Tjardes T, Simanski C, Bouillon B. Red-blood-cell to plasma ratios transfused during massive transfusion are associated with mortality in severe multiple injury: a retrospective analysis from the Trauma

Registry of the Deutsche Gesellschaft fur Unfallchirurgie. *Vox sanguinis.* 2008;95(2):112-119.

16. Teixeira PG, Inaba K, Shulman I, et al. Impact of plasma transfusion in massively transfused trauma patients. *The Journal of Trauma.* 2009;66(3):693-697.

17. Zink KA, Sambasivan CN, Holcomb JB, Chisholm G, Schreiber MA. A high ratio of plasma and platelets to packed red blood cells in the first 6 hours of massive transfusion improves outcomes in a large multicenter study. *American journal of surgery.* 2009;197(5):565-570; discussion 570.

18. Johansson PI, Ostrowski SR, Secher NH. Management of major blood loss: an update. *Acta Anaesthesiol Scand.* 2010;54(9):1039-1049.

19. Kutcher ME, Kornblith LZ, Narayan R, et al. A Paradigm Shift in Trauma Resuscitation: Evaluation of Evolving Massive Transfusion Practices. *JAMA surgery.* 2013.

20. Aucar JA, Sheth M. The storage lesion of packed red blood cells affects coagulation. *Surgery.* 2012;152(4):697-702; discussion 702-693.

21. Orlov D, Karkouti K. The pathophysiology and consequences of red blood cell storage. *Anaesthesia.* 2015;70 Suppl 1:29-e12.

22. Schubert P, Devine DV. Towards targeting platelet storage lesion-related signaling pathways. *Blood transfusion = Trasfusione del sangue.* 2010;8 Suppl 3:s69-72.

23. Bennett-Guerrero E, Veldman TH, Doctor A, et al. Evolution of adverse changes in stored RBCs. *Proc Natl Acad Sci U S A.* 2007;104(43):17063-17068.

24. Aubron C, Nichol A, Cooper DJ, Bellomo R. Age of red blood cells and transfusion in critically ill patients. *Annals of intensive care.* 2013;3(1):2.

25. Vraets A, Lin Y, Callum JL. Transfusion-associated hyperkalemia. *Transfus Med Rev.* 2011;25(3):184-196.

26. Patel MB, Proctor KG, Majetschak M. Extracellular ubiquitin increases in packed red blood cell units during storage. *J Surg Res.* 2006;135(2):226-232.

27. Tsukamoto T, Chanthaphavong RS, Pape HC. Current theories on the pathophysiology of multiple organ failure after trauma. *Injury.* 2010;41(1):21-26.

28. Lord JM, Midwinter MJ, Chen YF, et al. The systemic immune response to trauma: an overview of pathophysiology and treatment. *Lancet.* 2014;384(9952):1455-1465.

29. Xiao W, Mindrinos MN, Seok J, et al. A genomic storm in critically injured humans. *J Exp Med.* 2011;208(13):2581-2590.

30. Cole E, Davenport R, Willett K, Brohi K. The burden of infection in severely injured trauma patients and the relationship with admission shock severity. *The journal of trauma and acute care surgery.* 2014;76(3):730-735.

31. Ingraham AM, Xiong W, Hemmila MR, et al. The attributable mortality and length of stay of trauma-related complications: a matched cohort study. *Ann Surg.* 2010;252(2):358-362.

32. Hemmila MR, Jakubus JL, Maggio PM, et al. Real money: complications and hospital costs in trauma patients. *Surgery.* 2008;144(2):307-316.

33. Seghatchian J, Samama MM. Massive transfusion: an overview of the main characteristics and potential risks associated with substances used for correction of a coagulopathy. *Transfus Apher Sci.* 2012;47(2):235-243.

34. Inflammation and the Host Response to Injury. 2011. http://www.gluegrant.org. Accessed April 4, 2014.

35. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Critical Care Medicine.* 1985;13(10):818-829.

36. Osler T, Baker SP, Long W. A modification of the injury severity score that both improves accuracy and simplifies scoring. *J Trauma.* 1997;43(6):922-925; discussion 925-926.

37. Kuhne CA, Ruchholtz S, Kaiser GM, Nast-Kolb D. Mortality in severely injured elderly trauma patients--when does age become a risk factor? *World J Surg.* 2005;29(11):1476-1482.

38. American College of Surgeons. Searching for Verified Trauma Centers. 2015; http://www.facs.org/search/trauma-centers. Accessed February 25, 2015.

39. Committee on Trauma ACoS. *Resources for Optimal Care of the Injured Patient 2014.* American College of Surgeons;2014.

40. Association for the Advancement of Automotive Medicine. Abbreviated Injury Scale: What is the Abbreviated Injury Scale? 2006; http://www.aaam1.org/ais/index.php. Accessed March 2, 2012.

41. O'Neill B, Zador P, Baker SP. Indexes of severity: underlying concepts--a reply. *Health Serv Res.* 1979;14(1):68-76.

42. Ringdal KG, Skaga NO, Hestnes M, et al. Abbreviated Injury Scale: not a reliable basis for summation of injury severity in trauma facilities? *Injury.* 2013;44(5):691-699.

43. Kim YJ. Injury severity scoring systems: a review of application to practice. *Nurs Crit Care.* 2012;17(3):138-150.

44. Galvagno SM, Jr., Thomas S, Stephens C, et al. Helicopter emergency medical services for adults with major trauma. *Cochrane Database Syst Rev.* 2013;3:CD009228.

45. Wong TH, Lumsdaine W, Hardy BM, Lee K, Balogh ZJ. The impact of specialist trauma service on major trauma mortality. *The journal of trauma and acute care surgery.* 2013;74(3):780-784.

46. Dewar DC, Tarrant SM, King KL, Balogh ZJ. Changes in the epidemiology and prediction of multiple-organ failure after injury. *The journal of trauma and acute care surgery.* 2013;74(3):774-779.

47. Brooks SE, Mukherjee K, Gunter OL, et al. Do models incorporating comorbidities outperform those incorporating vital signs and injury pattern for predicting mortality in geriatric trauma? *J Am Coll Surg.* 2014;219(5):1020-1027.

48. American Association of Blood Banks. Circular of Information for the Use of Human Blood and Blood Components. In: American Association of Blood Banks, ed2013.

49. American Association of Blood Banks. Billing for Blood and Transfusion Services: Frequently Asked Questions and Answers. 2015;

http://www.aabb.org/advocacy/reimbursementinitiatives/Pages/billingfaq082907.aspx. Accessed March 6, 2015.

50. Arya RC, Wander G, Gupta P. Blood component therapy: Which, when and how much. *Journal of anaesthesiology, clinical pharmacology.* 2011;27(2):278-284.

51. Peiniger S, Nienaber U, Lefering R, et al. Balanced massive transfusion ratios in multiple injury patients with traumatic brain injury. *Crit Care.* 2011;15(1):R68.

52. Inflammation and the Host Response to Injury. *Infectious and Non-Infectious Complications - Definitions from the Trauma-Related Database (TRDB).* gluegrant.org 10/22/2007 2007.

53. Dewar DC, White A, Attia J, Tarrant SM, King KL, Balogh ZJ. Comparison of postinjury multiple-organ failure scoring systems: Denver versus Sequential Organ Failure Assessment. *The journal of trauma and acute care surgery.* 2014;77(4):624-629.

54. Sauaia A, Moore EE, Johnson JL, Ciesla DJ, Biffl WL, Banerjee A. Validation of postinjury multiple organ failure scores. *Shock.* 2009;31(5):438-447.

55. Gannon CJ, Pasquale M, Tracy JK, McCarter RJ, Napolitano LM. Male gender is associated with increased risk for postinjury pneumonia. *Shock.* 2004;21(5):410-414.

56. Magnotti LJ, Fischer PE, Zarzaur BL, Fabian TC, Croce MA. Impact of gender on outcomes after blunt injury: a definitive analysis of more than 36,000 trauma patients. *J Am Coll Surg.* 2008;206(5):984-991; discussion 991-982.

57. Berry C, Ley EJ, Tillou A, Cryer G, Margulies DR, Salim A. The effect of gender on patients with moderate to severe head injuries. *J Trauma.* 2009;67(5):950-953.

58. Lelubre C, Vincent JL. Relationship between red cell storage duration and outcomes in adults receiving red cell transfusions: a systematic review. *Crit Care.* 2013;17(2):R66.

59. Weinberg JA, MacLennan PA, Vandromme-Cusick MJ, et al. The deleterious effect of red blood cell storage on microvascular response to transfusion. *The journal of trauma and acute care surgery.* 2013;75(5):807-812.

60. Brown JB, Cohen MJ, Minei JP, et al. Debunking the survival bias myth: characterization of mortality during the initial 24 hours for patients requiring massive transfusion. *The journal of trauma and acute care surgery.* 2012;73(2):358-364; discussion 364.

61. Holcomb JB, Zarzabal LA, Michalek JE, et al. Increased platelet:RBC ratios are associated with improved survival after massive transfusion. *J Trauma.* 2011;71(2 Suppl 3):S318-328.

62. Cole E, Davenport R, De'Ath H, Manson J, Brockamp T, Brohi K. Coagulation system changes associated with susceptibility to infection in trauma patients. *The journal of trauma and acute care surgery.* 2013;74(1):51-57; discussion 57-58.

63. Bell TM, Bayt DR, Zarzaur BL. "Smoker's Paradox" in Patients Treated for Severe Injuries: Lower Risk of Mortality After Trauma Observed in Current Smokers. *Nicotine Tob Res.* 2015.

64. Ferro TN, Goslar PW, Romanovsky AA, Petersen SR. Smoking in trauma patients: the effects on the incidence of sepsis, respiratory failure, organ failure, and mortality. *J Trauma.* 2010;69(2):308-312.

65. Scolaro JA, Schenker ML, Yannascoli S, Baldwin K, Mehta S, Ahn J. Cigarette smoking increases complications following fracture: a systematic review. *J Bone Joint Surg Am.* 2014;96(8):674-681.

66. Arnson Y, Shoenfeld Y, Amital H. Effects of tobacco smoke on immunity, inflammation and autoimmunity. *Journal of Autoimmunity.* 2012;34(3):258-265.

67. Lao XQ, Jiang CQ, Zhang WS, et al. Smoking, smoking cessation and inflammatory markers in older Chinese men: The Guangzhou Biobank Cohort Study. *Atherosclerosis.* 2009;203(1):304-310.

68. Lee J, Taneja V, Vassallo R. Cigarette smoking and inflammation: cellular and molecular mechanisms. *J Dent Res.* 2012;91(2):142-149.

69. Rom O, Avezov K, Aizenbud D, Reznick AZ. Cigarette smoking and inflammation revisited. *Respiratory physiology & neurobiology.* 2013;187(1):5-10.

70. Hurley LL, Taylor RE, Tizabi Y. Positive and negative effects of alcohol and nicotine and their interactions: a mechanistic review. *Neurotoxicity research.* 2012;21(1):57-69.

71. Yaghoubian A, Kaji A, Putnam B, De Virgilio N, De Virgilio C. Elevated blood alcohol level may be protective of trauma patient mortality. *Am Surg.* 2009;75(10):950-953.

72. Zeckey C, Dannecker S, Hildebrand F, et al. Alcohol and multiple trauma: is there an influence on the outcome? *Alcohol.* 2011;45(3):245-251.

73. Molina PE, Sulzer JK, Whitaker AM. Alcohol abuse and the injured host: dysregulation of counterregulatory mechanisms review. *Shock.* 2013;39(3):240-249.

74. Melvan JN, Mooney J, Bagby GJ, Hunt JP, Batson R, Greiffenstein P. Drug and alcohol use complicate traumatic peripheral vascular injury. *The journal of trauma and acute care surgery.* 2013;75(2):258-265.

75. Ray RI, Aitken SA, McQueen MM, Court-Brown CM, Ralston SH. Predictors of poor clinical outcome following hip fracture in middle aged-patients. *Injury.* 2014.

76. Crutcher CL, 2nd, Ugiliweneza B, Hodes JE, Kong M, Boakye M. Alcohol intoxication and its effects on traumatic spinal cord injury outcomes. *J Neurotrauma.* 2014;31(9):798-802.

77. Mica L, Furrer E, Keel M, Trentz O. Predictive ability of the ISS, NISS, and APACHE II score for SIRS and sepsis in polytrauma patients. *European journal of trauma and emergency surgery : official publication of the European Trauma Society.* 2012;38:665-671.

78. Cuenca AG, Gentile LF, Lopez MC, et al. Development of a genomic metric that can be rapidly used to predict clinical outcome in severely injured trauma patients. *Crit Care Med.* 2013;41(5):1175-1185.

79. Haider AH, Villegas CV, Saleem T, et al. Should the IDC-9 Trauma Mortality Prediction Model become the new paradigm for benchmarking trauma outcomes? *The journal of trauma and acute care surgery.* 2012;72(6):1695-1701.

80. Kahloul M, Bouida W, Boubaker H, et al. Value of anatomic and physiologic scoring systems in outcome prediction of trauma patients. *European Journal of Emergency Medicine.* 2014;21(2):125-129.

81. Elson C. *Inflammation and Host Response to Injury Glue Grant defines MODS (multiple organ dysfunction syndrome, Denver criterion) when Denver Score >/= 3.* Massachusetts General Hospital; 2007.

Chapter Six

1. World Health Organization. Health Topics: Injuries. 2015; http://www.who.int/topics/injuries/en/. Accessed February 22, 2015.
2. Orlov D, Karkouti K. The pathophysiology and consequences of red blood cell storage. *Anaesthesia.* 2015;70 Suppl 1:29-e12.
3. Marik PE, Corwin HL. Efficacy of red blood cell transfusion in the critically ill: a systematic review of the literature. *Critical Care Medicine.* 2008;36(9):2667-2674.
4. Bohmer AB, Just KS, Lefering R, et al. Factors influencing lengths of stay in the intensive care unit for surviving trauma patients: a retrospective analysis of 30,157 cases. *Crit Care.* 2014;18(4):R143.
5. Hallet J, Lauzier F, Mailloux O, et al. The use of higher platelet: RBC transfusion ratio in the acute phase of trauma resuscitation: a systematic review. *Crit Care Med.* 2013;41(12):2800-2811.
6. Brown JB, Cohen MJ, Minei JP, et al. Debunking the survival bias myth: characterization of mortality during the initial 24 hours for patients requiring massive transfusion. *The journal of trauma and acute care surgery.* 2012;73(2):358-364; discussion 364.
7. Mitra B, Mori A, Cameron PA, Fitzgerald M, Paul E, Street A. Fresh frozen plasma (FFP) use during massive blood transfusion in trauma resuscitation. *Injury.* 2010;41(1):35-39.
8. Flatt JF, Bawazir WM, Bruce LJ. The involvement of cation leaks in the storage lesion of red blood cells. *Frontiers in physiology.* 2014;5:214.

9. Viswanathan M, Berkman ND. Development of the RTI item bank on risk of bias and precision of observational studies. *J Clin Epidemiol.* 2012;65(2):163-178.

10. Cripps MW, Kutcher ME, Daley A, et al. Cause and timing of death in massively transfused trauma patients. *The journal of trauma and acute care surgery.* 2013;75(2 Suppl 2):S255-262.

VITA

Educational Background

Year	Degree	Institution
2012	Masters of Science in Nursing	University of Kentucky, Lexington, KY
2006	Bachelors of Science Nursing	University of Kentucky, Lexington, KY

Professional Positions Held

Dates	Institution and Location	Position
May 2014 – Present	University of Kentucky College of Nursing, Lexington, KY	Graduate Research Associate, Dr. Jennifer Hatcher
Aug 2014 – Present	University of Kentucky College of Nursing, Lexington, KY	Graduate Research Associate, Dr. Elizabeth Salt
May 2011 – Dec 2011	University of Kentucky Chandler Medical Center Emergency Department, Lexington, KY	Staff nurse
Aug 2010 – May 2011	University of Kentucky College of Nursing, Lexington, KY	Graduate Teaching Assistant, Family Health Promotion and

		Communication Across the Lifespan, NUR 861, 8.0 hours
Jan 2010 – Dec 2013	University of Kentucky College of Nursing, Lexington, KY	Graduate Research Associate, Dr. Jennifer Hatcher
Jan 2010 – May 2010	Florence Crittenton Home, Lexington, KY	Staff nurse
May 2009 – April 2010	University of Kentucky Marketing Department and Pediatric Triage, Lexington, KY	Staff nurse
June 2006 – July 2009	University of Kentucky Chandler Emergency Department, Lexington, KY	Staff nurse

Scholastic and Professional Honors

Dorothy Luther Fellowship Award	2014
Emergency Nurses Association/Sigma Theta Tau International Research Award	2014
Nominated by University of Kentucky College of Nursing faculty for Dissertation Year Fellowship	2014
Sigma Theta Tau International Delta Psi Chapter Nursing Research	2014

Award	
UK College of Nursing Alumni Association President's Award – Outstanding MSN Student	2013
Nominated by University of Kentucky College of Nursing faculty for the University of Kentucky Presidential Fellowship for the 2013 academic year	2013
Pamela Stinson Kidd Scholarship	2011 and 2012

Professional Publications

Jones, A.R. & Frazier, S.K. 2015. 20 things you didn't know about blood transfusion. *Journal of Cardiovascular Nursing; 30*(1): 8-12.

Jones, A.R. & Frazier, S.K. 2014. Increased mortality in adult patients with major trauma transfused with blood components compared with whole blood. *Journal of Trauma Nursing; 21*(1): 22-29.